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An International Journal of Genetics

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HEREDITY VOLUME 14 PARTS 1 & 2 FEBRUARY 1960

THE INFLUENCE OF THE MATERNAL ENVIRONMENT ON GROWTH IN MICE

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1. INTRODUCTION

During the last fifty years many reports and essays have appeared concerning the inheritance of body size in mammals and several reviews of these studies are now available (Venge, 1950 ; Grüneberg, 1952). An examination of these and of the detailed reports on which they are based shows that the inheritance of body size has been examined in three main ways, *i.e.*

1. The inbreeding and crossbreeding of animals of diverse size.
2. The continued selection of animals of large and small size from a common parent stock.
3. The analysis of pedigree records to establish the genetic and environmental components of the phenotypic variation.

Each of these techniques has produced data leading to the conclusion that body size is determined by many genes in the manner common to other quantitative characters. Following upon this general conclusion many studies of body size have been concerned with the development of a reasonable theory of quantitative inheritance, and to this end they have been directed towards a comparison of observed experimental results with expectation as determined by theory. By such means the first concept of simple additive gene effects was extended to one in which dominance relations were included thus allowing of an explanation of heterosis and inbreeding depression in terms of homozygous and heterozygous allelic pairs.

But in spite of these advances many complications remain in the interpretation of experimental data on body size. Some of these such as scale effects and changes in the degree of dominance with selection have been discussed in detail (Mather, 1949 ; Fisher, 1930). The importance of many others is still obscure. For example, in the treatment of the data recorded in many experiments a number of simplifying assumptions have had to be made. Those commonly encountered include the absence of cytoplasmic and perhaps maternal effects, lack of interactions between the genotype and environment, the stability of environmental conditions for successive generations and the lack of a correlation between the genotype and environment.

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Of the factors known to be associated with mammalian growth, maternal effects appear to be of widespread importance. They have been reported to influence the growth of horses (Walton and Hammond, 1938), cattle (King and Donald, 1955 ; Brumby and Hancock, 1956), sheep (Hunter, 1956), rabbits (Venge, 1953) and mice (Bateman, 1954). Although of widespread occurrence the manner in which the maternal effect influences growth is far from clear. The possible mechanisms that have been suggested to explain the effect include cytoplasmic inheritance, nutrition, and endocrine factors (for review, see Hunter, 1956).

The importance of maternal effects on mammalian growth emphasises a particular problem encountered when selection experiments are undertaken, namely that when a selection response is observed there is a probable consequent change in the maternal environment provided for the next generation. It may be argued that by selecting within the litters of multiparous animals it is possible to avoid directly selecting for maternal environment but the problem of a possible genetic correlation between the character selected and the subsequent maternal performance then arises. Similarly it is apparent that an examination of inbreeding depression and heterosis in mammals is greatly complicated by differences in the maternal environment provided for different matings.

The experiments discussed here were intended to investigate the importance of the maternal environment to the growth of a large and small strain of mice selected by Falconer from a common base population, and further, to endeavour to clarify the nature of the maternal influence operating.

The experimental programme planned was made possible by the recent successful development of techniques of egg transplantation in mice (for reviews, see McLaren and Michie, 1956). The use of this technique enabled the prenatal maternal environment to be varied at will.

In brief, an attempt was made to answer the questions :

1. Are maternal effects of importance in explaining the asymmetrical selection response in body weight recorded by Falconer (1955) ?
2. In what manner are these maternal effects related to body size ?
3. What is the possible nature of the mechanism involved ?

2. MATERIALS AND METHODS

(1) *Stocks used*

The large and small strains of mice used in this work originated from the same base population formed by crossing four highly inbred strains (CBA, RIII, A and C57BL). Selection for body weight at six weeks of age was made within litters for some forty generations in the upward direction and thirty generations in the downward direction. At generation 31 in the up line and generation 20 in the down

lines reverse selection lines were started. In the small line this resulted in an immediate response and was accompanied by an increase in fecundity and a decline in the variability of body weight (Falconer, 1955). In the large line the response to reverse selection was slower (Falconer, unpublished). The parental lines chosen were the large strain animals from generations 37 and 38, and the reverse small strain animals from generations 30 and 31. The reverse selected small line animals were chosen rather than the small line animals because of their fecundity and lower variability.

The unselected stock originated from a cross of several heterogeneous strains. This cross had been maintained for eighteen generations with minimum inbreeding and without conscious selection for any character. The three stocks will be referred to as the large (L), small (S) and unselected (U), strains. In all three strains litters were weaned at 21 days after birth.

(ii) *Egg transfer*

Immature female mice aged 22-25 days were used as donors. Ovulation was induced by treatment with follicle stimulating and luteinising hormone. Three I.U. of F.S.H. (Serum Gonadotrophin B.P. Organon) were used as the priming dose followed by 3 I.U. of L.H. (Chorionic Gonadotrophin B.P. Organon) 48 hours later. Ovulation is believed to occur some 12 hours thereafter (Runner and Palm, 1953). The occurrence of mating was detected by the presence of a vaginal plug on the following morning. Three days later the mated donors were killed, the uterine horns dissected out and washed through with a small volume of Ringer phosphate saline (Pannett and Compton, 1924). The eggs, usually in the early blastocyst stage, were collected in a watchglass and identified under a binocular.

Recipient mice of the large and small strain were primed with F.S.H. and L.H. in exactly the same manner as the donor mice, then mated to a vasectomised male. Recipients of the unselected strain, owing to the much larger number of females available, were mated to vasectomised males and those found with plugs on any given day used as recipients. All egg transfers were made into recipients 2½ days after they were mated, for McLaren and Michie (1956) reported a better conception rate using 2½ day recipients rather than fully synchronised donors and recipients.

Recipient animals were anæsthetised with ether and a dorsal skin incision made over the region of the right ovary. The abdominal wall was then opened and the ovarian fat pad, ovary and Fallopian tubes exteriorised. Slightly below the tube-uteral junction an incision was made in the uterus with a needle and through this the end of a fine pipette carrying the eggs was inserted. In this manner approximately 10-15 eggs were inserted into the right uterine horn of each recipient. The ovary and fat pad were then returned to the abdominal cavity and the skin incision closed with a cotton suture.

(iii) *Analysis of growth data*

The weight of individual animals at a given age was influenced by a number of components of which genotype, maternal effect and litter size were the most important. Of these three major sources of variation, litter size was of little interest and added an unnecessary complication to the interpretation of results. From an experimental viewpoint it was impossible to standardise completely the size of litters, but by statistical manipulation the same end was aimed at. Each mean weight and variance was adjusted to that equivalent to a litter size of 5 animals.

Details of the analysis are as follows :

Analyses of variances, and of the covariance of the mean weight of litters and litter size, were made on birth weights, then on weekly weights to 6 weeks of age, thereafter at 8, 10 and 12 weeks of age. Separate analyses were performed for male and female mice after 3 weeks of age. In each analysis the error variance and group mean were adjusted to a mean litter size of 5. Then the mean of the separate male and female mice was estimated and the male and female error variance combined. From this combined error variance for each separate experimental group

of mice a pooled error variance and an average standard error for the group means was computed. From this the approximate difference required for significance between any two groups was estimated.

Approximately 10 litters were produced in each experimental group for it was estimated that with an average litter size of 5 and a coefficient of variation of the body weights of the order of 15 per cent., group sizes of this magnitude would provide sufficient material to detect ($P<0{\cdot}05$), with a probability of 75 per cent. differences of the order of 10 per cent. or more in mean body weight (Snedecor, 1956).

(iv) *Experiments performed and notation used*

As already pointed out, the letters L, S and U were used to denote the large, small and unselected strains respectively. To describe each experimental group a minimum of three letters was used, *e.g.* *S*/L/*U*.

The first letter indicates the strain of embryo implanted in the female, the second the strain of female in which the embryos were implanted and the third the strain of female which suckled the young after birth. When a transplantation or fostering took place the appropriate letter is italicised. For example, the above three letters indicate that small strain eggs were implanted in large strain females and the resulting young were fostered onto U strain females which reared them. Where crosses were made the female member is noted first.

Fifteen groups in all were compared in the course of six separate experiments. For convenience to the reader each experiment is tabulated below with a symbolised representation of the groups compared.

1. The influence of transplantation of fertilised eggs upon the subsequent growth of the resulting mice.

U/U/U and U/U/U

2. The influence of fostering within strains upon the weaning weight,
   (*a*) S/S/*S* and S/S/S
   (*b*) L/L/*L* and L/L/L
3. The relative importance of maternal effects in the large and small strains,
   (*a*) *U*/L/L and *U*/S/S
   (*b*) *S*/L/L and S/S/S
   (*c*) *L*/S/S and L/L/L
4. The relationship of the maternal performance to body size,
   (*a*) U/U/U, *U*/L/L, *U*/S/S
   (*b*) *L*/U/U and L/L/L
   (*c*) *S*/U/U and S/S/S
5. The partitioning of the prenatal and postnatal maternal environment,
   (*a*) S×L/S/S, S×L/S/U and *S*×*L*/U/U
   (*b*) S/S/*L* and S/S/S
   (*c*) L/L/*S* and L/S/L
   (*d*) S/S/*U* and S/S/S
   (*e*) U/U/*S* and U/U/U
6. The role of cytoplasmic inheritance and sex linkage in the determination of body size,
   (*a*) *S*×*L*/U/U and *L*×*S*/U/U
   (*b*) S×L/S/S and L×S/L/L

3. RESULTS

(i) *The influence of transplantation of fertilised eggs upon the subsequent growth of the resulting mice*

The work of Gates (1956) established that fertilised eggs obtained from immature mice as a result of treatment with gonadotrophins were viable and capable of normal development. It was also observed

that the transplantation of $3\frac{1}{2}$ day mouse eggs did not appreciably affect their embryonic weight at 18 days. This study did not, however, include the postnatal growth phase of the young resulting from transferred eggs, nor was anything known of the impact of the transplantation procedure upon the postnatal maternal performance of the host female. For these reasons it was considered desirable to compare the postnatal growth of embryos resulting from egg transplants with that of normal native embryos. Fertilised eggs from immature U strain mice were transplanted to mature $2\frac{1}{2}$ day pseudo-pregnant females of the same strain and the consecutive weights of the resulting embryos compared with those of embryos of the U strain conceived and born in the normal manner. The relevant growth data for this comparison are presented in lines 1 and 2 of table 1.

No difference between the two groups was apparent at any stage of growth. Although this comparison was made in the U strain only, it appears reasonable to extend the conclusion that transplantation *per se* is without effect on the subsequent growth potential of the embryo to the large and small strains as well.

(ii) *The influence of fostering within strain upon the weaning weight*

At birth many litters were cross-fostered to females of another strain, the rationale for which rested on the hypothesis that cross-fostering is without detrimental effects to subsequent growth rates. The evidence available concerning this question appeared to be confined to two reports. In 1950 Butler and Metrakos produced data suggesting that fostering had a detrimental effect on pre-weaning growth, though the data available were limited. Conversely, Bateman (1954) reported that fostering *per se* had no influence upon the 12 day weight of suckling mice.

In view of the discrepancy between the conclusions of these two reports it was considered advisable to investigate the problem in the stocks used in this work.

Table 2 lists the weaning weights of control and fostered litters, these being subdivided into litter sizes. As no systematic difference existed between the means or variance of litters of the same size within the two groups, it was concluded that the influence of fostering *per se* is not an appreciable source of variation when considering the weight increments of the large and small strains of mice.

Two other conclusions may be drawn from this table :

1. Litter size does not appear to influence the amount of variation within the litters.
2. The within litter variation and coefficient of variation is greater in the large strain than in the small. Expressed as a percentage of the total variation, however, the within litter variation of the large strain accounts for only 10 per cent. of the total variation whereas the within litter variation of the small strain accounts for 27 per cent. of the total variation.

TABLE 1

Summary of body weights (in gm.) adjusted to a mean litter size of 5 young

Group	Mean litter size	No. of Individuals	Week 0	1	2	3	4	5	6	8	10	12
1. U/U/U	5·9	59	1·69	4·81	7·47	10·63	16·99	22·29	24·62	27·32	28·92	30·22
2. *U*/U/U	5·75	46	1·79	4·88	7·53	10·53	16·02	21·46	24·09	27·11	28·79	29·96
3. *U*/S/S	3·77	49	1·43	3·78	6·16	8·86	14·96	19·36	21·73	24·20	25·34	26·67
4. *U*/L/L	4·0	40	1·70	4·75	7·63	10·73	17·14	21·37	24·28	27·32	28·89	29·84
5. S/S/S	4·5	44	1·16	3·23	5·41	6·70	9·28	12·00	13·45	15·04	15·81	16·64
6. *S*/L/L	4·1	37	1·31	3·65	5·81	7·42	10·05	12·02	13·84	15·85	17·36	18·50
7. L/L/L	6·1	55	1·58	4·76	7·34	9·73	16·67	24·24	27·61	29·98	32·02	33·27
8. *L*/S/S	3·0	32	1·36	3·46	5·48	7·55	14·37	22·00	24·89	28·75	30·82	32·86
9. *S*/U/U	5·8	58	1·43	4·32	6·88	8·76	13·03	15·55	16·72	18·94	20·57	21·88
10. *L*/U/U	3·3	31	1·74	5·28	8·33	11·42	20·69	26·14	28·70	31·68	33·76	35·05
11. *S*×*L*/U/U	4·3	43	1·66	4·65	7·71	9·92	16·29	21·36	23·57	26·24	27·71	29·37
12. *L*×*S*/U/U	5·25	63	1·58	4·29	6·99	9·70	14·97	19·57	21·79	24·00	25·68	27·09
13. S×L/S/S	4·80	48	1·24	3·65	6·22	9·07	14·80	19·45	21·41	23·62	25·33	26·52
14. L×S/L/L	6·67	60	1·50	3·76	6·54	8·96	13·42	18·74	20·94	23·83	25·71	26·91
15. S×L/S/U	5·20	52	1·29	4·66	8·10	10·58	16·27	20·94	22·52	24·62	26·62	28·18
Pooled regression of weight on litter size within groups			−0·039	−0·154	−0·401	♀ only −0·607 ♂ only −0·620	−0·698	−0·547 −0·652	−0·523 −0·659	−0·508 −0·626	−0·506 −0·634	−0·496 −0·693
Pooled error mean square within groups corrected for litter size			0·028	0·298	1·356	2·669	7·034	8·364	6·636	7·139	8·222	8·800
Approx. difference required for significance between any two groups (P. 0·05)			0·13	0·41	0·87	1·23	1·97	2·15	1·85	1·99	2·13	2·21

(iii) *The relative importance of maternal effects in the large and small strains*

As already pointed out in the introduction, Falconer selected these large and small strains of mice using within litter selection, the criteria of selection being the deviation of each individual from the mean value of the family to which it belonged. Assuming random drift to be small, it follows that any difference in the maternal environment provided by the two selected lines must be a consequence of a correlation between body size and maternal environment.

An appraisal of the difference in the maternal environment of the two lines was made in two ways. In the first experiment fertilised eggs

TABLE 2

A comparison of weaning weights of normal and fostered litters of the small and large strains

Litter size	Small strain						Large strain					
	Control			Fostered			Control			Fostered		
	N	W	V	N	W	V	N	W	V	N	W	V
2	20	8·72	0·248	10	8·91	0·578	...	...	...	...	...	...
3	30	8·34	0·562	21	8·74	0·160	30	11·5	1·134	6	13·32	0·263
4	40	7·93	0·120	28	7·83	0·228	40	12·0	0·724	20	12·37	1·296
5	50	7·76	0·298	45	7·46	0·263	50	10·91	0·571	30	10·82	1·537
6	60	7·70	0·283	42	7·22	0·438	60	10·28	0·680	24	10·26	1·192
7	70	7·06	0·392	56	6·86	0·537	70	9·95	1·160	28	11·17	0·699
8	...	...	...	...	...	...	80	8·88	0·628	32	9·01	0·569
Components of variance :												
Between size		0·43				0·44			4·73			5·55
Between litters		0·43				0·44			3·22			2·05
Within litters		0·35				0·40			0·86			0·98

N = Number of individuals. W = Mean weight in gm. V = Variance within litters.

of the U strain were implanted in both large and small strain females, and the resulting young compared in growth rate. In the second experiment fertilised eggs of the small strain were implanted in large strain mothers, while fertilised eggs of the large strain were implanted in small strain mothers. The subsequent growth of the embryos was compared with that of normally born large and small strain mice. The results of the first experiment are presented in lines 3 and 4 of table 1 and in the graphs marked *U*/L/L and *U*/S/S of fig. 1 (*a*). The results of the second experiment are presented in lines 5 and 6, and 7 and 8 of table 1, and in the remaining graphs of fig. 1 (*a*).

The results of the first experiment indicate beyond all doubt that a substantial difference existed between the maternal environment provided by the two strains. A large difference in weight was already

apparent at birth, a difference which steadily increased up to 8 weeks of age at which stage it appeared relatively stable.

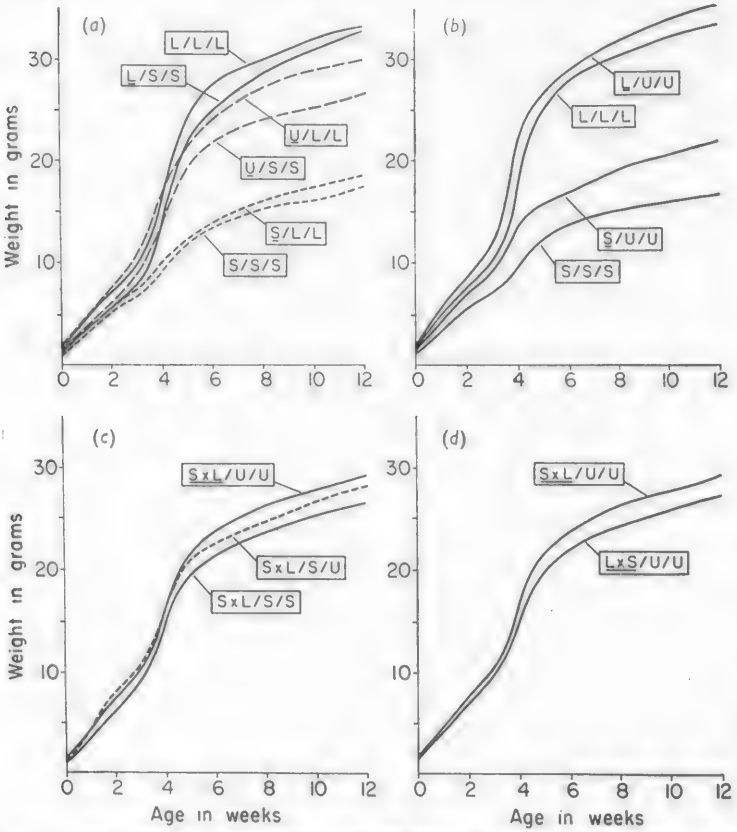

FIG. 1.—(*a*) Joint effects of prenatal and postnatal environment. Growth curves of young of the three strains when reared in and suckled by L-strain or by S-strain females. (*b*) Joint effects of prenatal and postnatal environment. Growth curves of L-strain and S-strain young when reared in and suckled by their own mothers or by U-strain females. (*c*) Separate effects of prenatal and postnatal environment. Growth curves of hybrid young (S ♀ × L ♂) when reared in and suckled by S-strain females (lower curve); when reared in S-strain females but suckled by U-strain females (middle curve); and when reared in and suckled by U-strain females (upper curve). (*d*) Difference in growth between reciprocal crosses of Large and Small strains when reared in and suckled by U-strain females.

The results of the second experiment substantiate those of the first and indicate that at least part of the difference in body weight observed between the large and small strain lines was due to a difference in the maternal environment provided by the two strains. Because of the

nature of the selection programme used in developing these stocks it would appear that this difference in maternal environment originated because of a change in the body weight of the selected parental stocks.

(iv) *The relationship of the maternal influence to body size*

The experiments described in the previous section indicated that a substantial difference existed in the maternal environment provided for the two strains. This difference was attributed to the change of body size produced by selection. The question remains whether or not this difference in the maternal environment is simply related to body size, for it might be supposed that while the small strain animals provide a poorer environment than the larger strain, the large strain animals might provide no better environment than that provided by mice unselected for size.

Some evidence for an asymmetrical maternal effect was provided by a comparison of large and small strain females as host mothres of U strain young (table 1, lines 2, 3 and 4). Reared in large strain host mothers, these U strain young grew at the same rate as those reared in their own U strain mothers, but reared in small strain host mothers they grew much more slowly. In other words, large strain females used as host mothers were equal in maternal performance to the U strain females; but small strain females used as host mothers recorded a much poorer performance. The comparison may be seen in the graphs marked *U*/L/L and *U*/S/S in fig. 1 (*a*). The graph of U/U/U was indistinguishable from *U*/L/L, and is not shown separately.

Further evidence was obtained by implanting both large and small strain eggs in U strain females and comparing the growth of the resultant embryos with the control stocks of the large and small strains. The results of this comparison are presented in table 1 (lines 5 and 9, and 7 and 10) and fig. 1 (*b*).

Rather surprisingly perhaps, both the large strain and the small strain animals were found to be greatly increased in size when implanted in U strain females, even though the U strain females were smaller in size than the large strain females. It follows that the maternal environment provided by the large strain females must be inferior to that provided by the U strain females when rearing large and small strain embryos. On the other hand, it was shown that the maternal performance of large strain females was equivalent to that of the U strain females when both were rearing U strain embryos. In other words, an interaction exists between the genotype of the embryo and the maternal environment provided. Two other conditions emerge from these results:

1. The difference in maternal environment produced by changes in body weight is asymmetrical.

2. Though body size and maternal effect are related, the fact that the maternal environment provided by the U strain stock is superior to that provided by the large strain stock indicates that there are factors associated with a good maternal environment that are unrelated to body size.

(v) *The partitioning of the prenatal and postnatal maternal environment*

The maternal environment provided by the female may be split into two major phases, *i.e.* the prenatal (the period from ovulation to parturition) and the postnatal (the period from parturition to weaning). A separation of the total maternal environment into these two phases is of considerable practical interest for though the prenatal phase is relatively difficult to influence save by severe changes in nutrition (Wallace, 1948) the postnatal period readily lends itself to environmental modification.

A partitioning of the maternal environment into the two phases was achieved in two separate experiments.

In the first experiment F_1 hybrids of small strain female, large strain male crosses were normally reared and compared to the same crosses fostered to U strain females. They were also compared to the same crosses implanted in and reared by U strain females. Three separate environments were thereby achieved, *i.e.* the normal, an alien postnatal, and an alien pre- and postnatal combined. Results for the growth of the three groups are given in table 1 (lines 11, 13, 15) and in fig. 1 (*c*).

As expected a difference in birth weight between the normal cross and those reared in U strain females was apparent. In the groups born of small strain females but reared by the U strain females this difference was quickly eliminated and did not again appear until the animals were 6 weeks of age. At this stage the weight of the animals implanted in U strain females surpassed that of those merely reared by U strain females. Throughout, the young mice born and reared by small strain females grew at a slower rate. From 6 to 12 weeks of age the relative difference between the three groups did not change appreciably, the position of the three groups suggesting that for this particular situation the postnatal environment accounted for about one-half of the total measurable maternal difference.

In the second series of experiments small and large strain embryos were mutually cross-fostered, as were small strain and U strain embryos, and weaning weights recorded. Limitations in the cage space available did not allow these animals to be retained beyond 3 weeks of age. The relevant weaning weights are tabulated in table 3.

The performance of small strain young reared by large strain females proved no better than that of small strain young reared by small strain females, although small strain young reared by U strain does were appreciably heavier at weaning. This observation suggested

that the large strain females do not markedly differ from the small strain in lactational capacity, from which it follows that the difference in maternal performance observed between the large and small strain must largely originate in the prenatal environment.

In contrast large strain young reared by small strain females were somewhat smaller at weaning than the large strain controls, an observation that suggested the small strain were actually inferior to the large strain in lactational capacity. Yet this conclusion appears untenable when the performance of U strain young reared by small strain females is considered, for the weaning weights of these U strain young were apparently normal.

TABLE 3

The influence of cross-fostering of small, large and unselected strains on body weight at 21 days

Litter size	Small strain						Large strain				U strain			
	Control		Suckled by L strain		Suckled by U strain		Control		Suckled by S strain		Control		Suckled by S strain	
	N	W	N	W	N	W	N	W	N	W	N	W	N	W
3	30	8·30	9	8·00	3	9·03	30	11·5	3	11·07	...	...	...	...
4	40	7·93	20	7·56	8	9·19	40	12·0	24	9·89	12	11·51	12	11·00
5	50	7·76	10	7·54	10	8·52	50	10·91	35	9·36	...	...		...
6	60	7·70	42	7·91	36	8·66	60	10·28	54	9·49	30	10·40	24	10·09
7	70	7·06	35	6·94	21	8·74	70	9·95	28	7·73	21	9·00	21	9·30
8	...	...	...	...		...	80	8·88	16	6·56	...	...	...	...
Components of variance :														
Between litters .	0·43		0·20		0·77		3·22		0·84		4·33		1·06	
Within litters .	0·35		0·31		0·67		0·86		0·60		0·68		0·43	

N = Number of individuals. W = Mean weight in gm.

From this apparent anomaly it appears that an interaction exists between the lactational performance of the female and the type of young being reared. But whatever the nature of such an interaction it may be concluded that the inferiority of the maternal performance of the small strain, for large strain embryos, appears to be determined in part by postnatal factors whereas the superiority of the large strain maternal performance for small strain animals appears to be almost solely determined by prenatal factors.

In general then it may be said that in each of the situations examined the prenatal maternal influence was of marked importance, whilst the postnatal contribution to the maternal performance varied according to the genotype of both the female and the young being suckled. This general conclusion is in agreement with that of Bateman (1954) who analysed the causes of variation in the 12-day weight

of mice. He found that the prenatal influence was greater than the postnatal influence while the combined total maternal influence (in litters of eight) amounted to 73 per cent. of the total variation present.

(vi) *The role of the cytoplasm and sex linkage in the determination of body size*

It is a fairly common observation that reciprocal crosses between mammals of different sizes lead to F_1 progeny that differ in size, the hybrid tending to resemble the size of the female rather than the male. There are three possible causes for the reciprocal difference: maternal effects, sex linkage and cytoplasmic inheritance.

The analysis of the role of sex linkage does not normally provide a particularly difficult problem. The first step of such an analysis involves a comparison of the reciprocals in the heterogametic sex; if these do not differ significantly then a sex linked difference is unlikely. The distinction between the maternal effect and the cytoplasmic influence is more difficult to make. The situation is complicated by possible differences in the cytoplasmic specificity, three types of which have been distinguished, *i.e.* specificity through ancestral continuity, through genetic conditioning in the egg stage, and through experimental change, *i.e.* dauermodification (Goldschmidt, 1955). Of these only the first may be considered as cytoplasmic heredity.

A distinction between the contribution of the collective cytoplasmic influence and the maternal environment may be made by standardising the maternal environment for each of the reciprocal crosses. This approach to the problem was used here.

Reciprocal crosses were made between the large and small strains and the resulting fertilised eggs transplanted to U strain females. The weights of the resulting young are presented in table 1 (lines 11, 12) and in fig. 1 (*d*).

At birth an appreciable difference in weight was apparent, in favour of the young resulting from the small females and large males. This difference persisted throughout the 12 weeks that body weights were recorded, resulting in a difference of weight of the order of 8 per cent. at 12 weeks of age.

Table 4 presents data for the body weights of the hybrid male and female mice, computed separately, and shows that the difference observed between the two reciprocal hybrids existed in the female mice as well as the males. Thus sex linkage does not appear to be the cause of the difference observed. Rather it appears that the cytoplasm of the small strain animals enhance body size to a greater degree than does the cytoplasm of the large strain.

As a consequence of this result reciprocal crosses were made between the large and small strains and allowed to develop and suckle normally. Growth data for these are tabulated in table 1 (lines 13, 14). A difference in birth weight reflecting differences in prenatal environment was apparent but on weaning at 21 days this difference was negligible.

Thereafter no apparent difference existed between the two crosses. The previous experiments recorded here established that the difference in maternal environment in the two strains would lead to the expectation that the large female, small male cross would actually be larger than its reciprocal, but this was not the case. This apparent

TABLE 4

Body weights of reciprocal F_1 hybrids of the large and small strains (gm.)

Cross	Age in weeks					
	4	5	6	8	10	12
$S \times L/U/U$ ♀	15·0	19·89	21·51	23·86	24·73	25·78
$L \times S/U/U$ ♀	14·31	17·89	19·70	21·55	22·76	23·89
$S \times L/U/U$ ♂	17·57	22·82	25·62	28·62	30·68	32·96
$L \times S/U/U$ ♂	15·97	21·25	23·87	26·45	28·60	30·29

anomaly might be explained in terms of the counter-balancing of the poorer maternal environment of the small strain by a greater cytoplasmic contribution of the small strain to growth.

4. DISCUSSION

(i) *Analysis of data*

In the treatment of the data presented, several simplifying assumptions were made without prior discussion of their validity. Some comment on these points is called for.

In the first place the relationship of body weight and litter size was treated as linear. Though this condition is not strictly true, the actual departure from linearity over the range of mean litter sizes considered, as indicated in table 2, is so small as to make this criticism of minor significance.

In the second place litter size has been taken as the number of living young the female reared beyond 24 hours, but because appreciable mortality occurred at the time of parturition this measure of litter size actually underestimates the true litter size. This approximation was made for two reasons :

1. The weight of young at any weighing prior to weaning was largely dependent upon the number of young being reared by the female at that period of time, rather than on the number of young born in the litter.
2. As there was no reason to believe marked differences occurred in the percentage postnatal loss of young within litters in the various groups it was considered unlikely that any serious bias would be introduced by using the 24-hour post-partum number of young.

The procedure of using the size of litter at 24 hours thus appeared a reasonable compromise between the two conflicting alternatives of number born and number reared.

The third query that may be raised concerns the validity of pooling regressions and variances within groups when there was prior evidence illustrated in table 2, suggesting that the variances of the large and small strains were different. The alternative to pooling the within group estimates was to use each separately in adjusting the group mean and its variance for a standard litter size of five young. As each group comprised approximately ten litters, a considerable amount of sampling variation entered into individual within group estimates. Thus it was argued that the pooling of the data would be less likely to bias the adjusted means and variances than by using individual group estimates. In fact the corrections applied to final body weights in each group were very small (about 0·5 gm.), while the differences of interest between the various groups were usually sufficiently clear cut to give a definite answer to the problem posed.

(ii) *Reproductive physiology*

An examination of various aspects of the reproductive physiology of the strains of mice used in the course of this work is not strictly relevant to the object of this study. Nevertheless, several points appear worthy of mention ; in particular, the recovery of fertilised ova, and the success achieved in causing these to implant.

The number of ova recovered from immature females following superovulation showed a marked difference between strains, small strain and U strain females providing many more eggs per female than large strain females. There was also an appreciable difference in the uniformity of development of these eggs at the time of recovery. Eggs from the small and U strains were usually in the blastocyst stage, whereas many large strain eggs were in the late morula stage and many others appeared to be fragmenting. Coupled with this problem of a lower available number of viable eggs from fertile matings of the large strain, males of this strain showed marked variability in their mating performance, many exhibiting little desire to mate with immature superovulated females. No trouble in this respect was experienced with U or small strain males.

The percentage of successful pregnancies resulting from egg transplantation was high when using U strain recipients ; about 80 per cent. of operations resulting in pregnancy. On the other hand small strain females proved refractory in this regard, for only 20 per cent. of transfer operations resulted in successful implantations. With both of these strains pregnancy was normally accompanied by successful parturition and lactation performance. This was not the case with the large strain recipients. Though the percentage of transplants resulting in pregnancy appeared satisfactory, *i.e.* about 60 per cent. of operations, the incidence of death at parturition was very high.

Many young appeared to be suffocated during the birth process and many others, both dead and alive, were eaten by the recipient female. Even amongst large strain females successfully littering, a number of litters up to a week of age were suddenly killed and eaten by the female for no obvious reason. This problem occurred to a lesser degree in the large strain parental stocks and entailed keeping a much larger parental stock than was envisaged in the original design of the experiment.

(iii) *The variation in maternal performance*

From the results of the egg transplants between the various strains four main conclusions emerge :

1. There is a difference in the maternal environment provided by the large and small strain which has resulted from changes in body size.
2. This difference in maternal environment between the two strains comes about mainly by a deterioration in the maternal performance of the small strain.
3. The genetic make-up of the embryo influences the maternal performance rating of the female, *i.e.* the embryo creates a specific demand both prenatally and postnatally.
4. A major portion of the maternal influence of the female on the growth of her young occurs during the prenatal period.

The problem remains of examining the possible mechanisms underlying these observations.

Perhaps the most surprising feature of the result of these experiments is the asymmetry of the change in maternal performance resulting from selection for body size and it is of considerable interest to enquire how it is that an increase in body size fails to increase maternal performance to the same degree as an equivalent decline in size decreases it.

Falconer (1955) sought to explain an asymmetry of the postnatal maternal performance in the following way. He suggested that there were two components in maternal performance, one related to anatomical development (*i.e.* size of mammary glands), the other to physiological efficiency. The anatomical component would be expected to be directly related to body size, whereas the physiological component would not. Rather, as this physiological component is in turn a component of natural fitness, it would show overdominance as postulated by Lerner (1954). An increase in homozygosis brought about by changes in gene frequency as a result of selection would then produce a decline of the physiological component in both lines. The result in the large line of the simultaneous changes in the anatomical and physiological components would be a counterbalancing of increased size and decreased lactational efficiency. In the small line there would be a decline in both size and lactational efficiency resulting in the large net decline of maternal performance observed.

As it stands this attractive explanation cannot be reconciled with the present situation for it was shown that the prenatal maternal effect was at least of equal importance to the postnatal. However, it seems possible that an analogous situation applies during the prenatal embryonic period. It may be argued that the anatomical component is represented by the size of the fœtal placenta, and the physiological component is represented by the efficiency of the placenta as an organ of interchange. If this were the case a close parallel of Falconer's explanation would be expected.

This hypothesis rests largely on two basic premises, (*a*) that embryo size and placenta size are related, (*b*) that a variation occurs in the functional efficiency of the combined maternal and fœtal placenta as an organ of interchange.

The literature available covering these phases of the physiology of the placenta has been reviewed in detail by Huggett and Hammond (1952). Suffice to state here that reasonable evidence establishing the validity of both points may be found therein, in which case it follows that the above explanation of the nature of the prenatal maternal effect offers a reasonable working hypothesis upon which further experimental work might be based.

(iv) *The cytoplasmic influence*

Though the difference that was established between the reciprocal crosses reared in the same environment provides apparent evidence that cytoplasmic factors are influencing growth, three further possible explanations may be invoked. The first lies in the fact that the eggs spent $3\frac{1}{2}$ days post-ovulation in their own dam prior to transplantation. Thus it may be argued that the difference observed in the reciprocal crosses is merely a consequence of this early maternal environment. Some evidence supporting this explanation is provided by the observation that the eggs of the small strain appeared slightly further developed at $3\frac{1}{2}$ days' post-ovulation than did those of the large strain. On the other hand it was shown that the post-implantation environment of the small strain was poorer than that of the large strain and it seems unlikely that the reverse would be true of the pre-implantation environment.

A further possible explanation lies in terms of differential mortality of eggs actually implanted but in view of the success achieved in causing eggs to implant in the U strain females this too seems unlikely. A third possibility is that it is merely a chance result. Obviously, further work on this point is required.

5. SUMMARY

1. The technique of ova transplantation was used in an investigation of the importance and nature of the maternal influence upon the growth of a large and small strain of mice. The strains of mice used had been

established by Falconer using within litter selection for approximately 35 generations.

2. Preliminary experiments established that neither transplantation nor fostering of young within strains influenced the growth potential of the embryos.

3. A marked difference was demonstrated in the maternal environment provided by the large and small strain females to embryos of a non-related unselected strain.

4. Compared to an unselected outbred strain both the large and small strain females proved inferior in maternal performance, but the main difference between the maternal performance of the large and small strains came about by a reduction in the maternal performance of the small strain.

5. An interaction between the prenatal maternal environment of the female and the genotype of the embryo implanted was apparent.

6. The partitioning of the total material environment into prenatal and postnatal phases, demonstrated the marked importance of the prenatal phase to growth. The postnatal contribution varied according to the genotype of both the female and the young being suckled. An interaction between the lactational performance of the female and the genotype of the young was apparent.

7. Sex linked genes were not responsible for any marked effect on body size, but evidence was found suggesting that the cytoplasmic influence on growth was greater in the small strain than in the large strain.

8. The results are discussed in relation to the interpretation of selection experiments.

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6. REFERENCES

BATEMAN, N. 1954. The measurement of milk production of mice through preweaning growth of suckling young. *Physiol. Zool.*, *27*, 163.

BRUMBY, P. J., AND HANCOCK, JOHN. 1956. A preliminary report of growth and milk production in identical and fraternal twin cattle. *N.Z. J. Sci. Tech. 38A*, 184.

BUTLER, L., AND METRAKOS, J. D. 1950. A study of size inheritance in the house mouse. I. The effects of milk source. *Can. J. Research, D*, *28*, 16.

FALCONER, D. S. 1955. Patterns of response in selection experiments with mice. *Cold Spr. Hbr. Symp.*, *XX*, 178.

FISHER, R. A. 1930. *The genetical theory of natural selection.* Oxford University Press.

GATES, ALLEN N. 1956. Viability and developmental capacity of eggs from immature mice treated with gonadotrophins. *Nature*, *177*, 754.

GOLDSCHMIDT, RICHARD B. 1955. *Theoretical Genetics.* University of California Press.

GRÜNEBERG, H. 1952. *The genetics of the mouse*. Martinus Nijhoff, The Hague.

HUGGETT, A. ST G., AND HAMMOND, J. 1952. Marshall's *Physiology of Reproduction*, chap. 16. Longmans.

HUNTER, G. L. 1956. The maternal influence on size in sheep. *J. Agric. Sci.*, *48*, 36.

KING, J. W. B., AND DONALD, H. P. 1955. A study of the variation in twin cattle. III. Growth. *J. Dairy Res.*, *22*, 1.

LERNER, I. M. 1954. *Genetic homeostasis*. Oliver and Boyd Ltd.

MCLAREN, ANNE, AND MICHIE, DONALD. 1956. Studies on the transfer of fertilised mouse eggs to uterine foster-mothers. I. Factors affecting the implantation and survival of native and transferred eggs. *J. exp. Biol.*, *33*, 394.

MATHER, K. 1949. *Biometrical genetics*. Methuen Press.

MOSSMAN, H. W. 1937. Comparative morphogenesis of the foetal membranes and accessory uterine structures. *Contr. Embryol. Carneg. Inst.*, No. 158, *26*, 129.

PANNETT, C. A., AND COMPTON, A. 1924. The cultivation of tissues in saline embryonic juice. *Lancet*, *1*, 381.

RUNNER, M. N., AND PALM, J. 1953. Transplantation and survival of unfertilised ova of the mouse in relation to post-ovulatory age. *J. exp. Zool.*, *124*, 303.

SNEDECOR, G. W. 1956. *Statistical methods*. Iowa State College Press.

VENGE, O. 1950. Studies of the maternal influence on the birth weight in rabbits. *Acta Zoologica*, Bd. *XXXI*.

VENGE, O. 1953. Studies of the maternal influence on the growth in rabbits. *Acta Agric. Scand.*, *III*, 243.

WALLACE, L. R. 1948. The growth of lambs before and after birth in relation to the level of nutrition. *J. Agric. Sci.*, *38*, 93-153, 243-407.

WALTON, A., AND HAMMOND, J. 1938. The maternal effects on growth and conformation in Shire horse-Shetland pony crosses. *Proc. Roy. Soc. Lond.*, Series B, *125*, 311.

AN ATTEMPT TO COMPARE THE FITNESS OF POLYMORPHIC AND MONOMORPHIC EXPERIMENTAL POPULATIONS OF *DROSOPHILA PSEUDOOBSCURA*

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1. INTRODUCTION

Chromosomal polymorphism, due to inversions of blocks of genes, is widespread in natural populations of many species of Drosophila. The polymorphism is probably almost always adaptive and usually balanced. Experimental populations which contain mixtures of karyotypes as a rule reach stable equilibria; this indicates that the structural heterozygotes are heterotic. The adaptive values of the karyotypes are, however, quite sensitive to environmental variations; the superior fitness of the heterozygotes may be more pronounced in some environments than in others, and it may even be absent altogether. It appears, however, that the heterozygotes possess net adaptive advantages over the range of the environments to which the population is exposed in its natural habitats.

Direct comparisons of the fitnesses of polymorphic and monomorphic populations have, however, seldom been attempted. This is the problem to which we address ourselves in the pages that follow. Third chromosomes with AR and with CH gene arrangements are common in many populations of *Drosophila pseudoobscura* in California and elsewhere. Dobzhansky (1948, 1957), Levine (1955), and Levene, Pavlovsky, and Dobzhansky (1958) have shown that experimental populations which contain these chromosomes are subject to strong selection which at 25° C. conduces to an equilibrium of about 70-75 percent AR and 25-30 percent CH. Taking the fitness of AR/CH heterozygotes to be unity, that of AR/AR homozygotes turns out to lie, in different experiments, between 0·65 and 0·85, and that of CH/CH homozygotes between 0·42 and 0·60. We have accordingly set up several experimental populations with AR and CH chromosomes in laboratory population cages. Three of these were polymorphic populations containing AR and CH chromosomes, two monomorphic populations with only AR, and two monomorphic populations with CH chromosomes. After several generations during which the populations might have undergone selection for adaptation to the environments of the population cages, attempts were made to obtain estimates of certain parameters which we believe to be significant for evaluation of the fitness of the populations.

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2. MATERIAL AND TECHNIQUES

The foundation stocks of the experimental populations were prepared using 16 strains homozygous for AR, and 13 strains homozygous for CH, all derived from flies collected in the locality of Pinon Flats, Mount San Jacinto, in California. The foundation stocks of the monomorphic AR and CH populations consisted of about 2000 flies each, obtained by intercrossing about equal numbers of flies from all the AR or CH strains respectively. The polymorphic populations were also descended from 2000 founders each, but were obtained by intercrosses of all the available AR and CH strains; the foundation stocks were so contrived that the initial gametic frequencies of AR and CH chromosomes were 0·2 and 0·8 respectively. The experimental populations were started on the following dates (all in 1957):

Population no.	Chromosomes	Date
173	AR+CH	January 10
174	CH	January 11
175	AR	February 1
176	AR+CH	February 5
180	AR	May 11
181	AR+CH	May 11
182	CH	May 11

All the populations were kept in wooden cages containing 15 food cups each, a fresh cup being inserted generally on alternate days. Nos. 174-182 were kept at 25° C. in well-regulated incubators provided with electric fans, and No. 173 at 25° but in a constant temperature room with poor ventilation. From time to time a cup with food in which pupæ were beginning to form was withdrawn from each of a series of cages (Nos. 173-176 or Nos. 180-182), attached by adhesive tape to a glass "chimney" having a diameter equal to that of the cup, provided with a cotton stopper and placed in an incubator at 25° C. When the adults began hatching in the cups, they were collected daily (including Sundays), etherised, counted, and weighed individually on a Misco Quartz Helix Balance, on which a load of 5 mg. caused an extension of 67 mm. from zero (read to one-half of a mm.). Only flies with fully expanded and hardened wings were weighed. It is important for the evaluation of the data in tables 2-6 to note that the samples from the different cages were not taken simultaneously. The series of populations Nos. 173-176 were sampled between 5th November 1957 and 9th January 1958, and again between 8th and 16th June 1958. The series of populations Nos. 180-182 were sampled between 30th January and 30th May, and again between 27th June and 15th July 1958. It will be seen that during most of 1958 the populations were in better condition than during 1957, as a consequence of which the cups yielded more flies in 1958 than in 1957.

The two series of populations are, thus, not necessarily comparable, while the populations within a series are, with the possible exception of No. 173 (see above).

At approximately the same time as each cup to be used for the determination of the biomass was taken from each of the populations, another similar cup was removed for the purpose of measuring wing length. All the flies were allowed to emerge from such a cup and a random sample taken from the total emergence in each cup. Ten samples (cups) were taken from each of populations 173, 176, 175 and 177 and five samples from populations 180-2. The number per sample was fifty flies of each sex, or the total emergence if this was less than fifty flies; no fly was rejected for any reason other than torn or crumpled wings. Both wings were then removed from each fly and mounted in paraffin oil under cover-glasses, ten pairs of wings to a slide, with the left wings forming a lower row and the right wings an upper row. The measurement of wing length was accomplished using a widefield binocular microscope at a magnification of 60×, with a standard eyepiece micrometer, divided into 100 divisions; wing lengths were estimated to

one-tenth of a scale division. The distance chosen to represent wing length was from a point near the posterior end of the humeral cross-vein to the tip of the wing near the end of the third vein, such distance in an average wing corresponding to about 85 units of the micrometer scale.

3. RESULTS

(i) *The selection process in the polymorphic populations*

The changes which took place in the three polymorphic populations are shown in table 1. The foundation stocks had 20 percent of AR and 80 percent of CH chromosomes. Control samples taken in two populations fourteen days after the start (*i.e.*, eggs from the surviving individuals of the foundation stocks) had, as expected, the initial

TABLE 1

Changes in the frequencies of AR and CH chromosomes in the polymorphic populations (in percent)

Days	173		176		181	
	AR	CH	AR	CH	AR	CH
0	20·0	80·0	20·0	80·0	20·0	80·0
14	22·7	77·3	20·7	79·3	...	...
35	39·3	60·7	40·0	60·0	40·7	59·3
70	45·0	55·0	48·3	51·7	41·3	58·7
105	50·0	50·0	50·3	49·7	55·3	44·7
140	60·7	39·3	56·7	43·4	...	...
175	57·3	42·7	...	...	...	...
264-275	66·3	33·7	66·3	33·7	67·7	32·3
302	64·3	35·7	...	...	...	...
365	64·3	35·7	71·3	28·7	67·7	32·3
488	...	...	...	...	69·0	31·0
583	...	...	78·3	21·7	...	...
610	72·7	27·3	...	...	...	...

frequencies unchanged. In the first generation developing in the cages, the frequency of AR chromosomes about doubled (see the 35th day sample in table 1; the mean generation length in these populations is estimated to be about 25 days). In about four generations from the start, the AR and CH chromosomes became equally frequent (the 105th day samples). A year after the start an equilibrium was approached but apparently not yet fully attained. The samples in which the flies were counted, weighed, and measured were, thus, taken during the period in the life of the polymorphic populations when the latter consisted of about 45-50 percent of AR/CH structural heterozygotes, and when the incidence of AR homozygotes was greater than that of CH homozygotes.

(ii) *Number of flies produced*

The means and variances of the numbers of the flies which hatched from the cups taken from the different population cages are reported

in table 2. The amounts of the food medium placed in the different cups were, of course, as uniform as practicable (about 25 cc.). The number of the eggs which the flies in the population cages deposit on each cup is probably always greater, and frequently greater by at least one order of magnitude, than the number of adult flies which eventually hatches. The number of the adults that hatch is, thus, an indication of the ability of the genotypes in a given population to utilise successfully the environmental opportunities at their disposal.

It can be seen at a glance that the first series of populations (Nos. 173-177) was less productive than the second series (Nos. 180-182). As stated above, this is ascribable to environmental conditions during the year 1958 being better than during 1957. What interests us are

TABLE 2

Number of flies hatching per cup in different populations

Population	Sex	Mean	Variance	Variation coefficient	Cups examined
173, AR+CH	Female	99·93	1629	40·4	33
176, AR+CH	Female	131·50	2265	36·2	33
175, AR	Female	86·24	1985	51·7	33
177, CH	Female	97·88	2781	53·9	33
181, AR+CH	Female	188·35	3383	30·8	20
180, AR	Female	139·75	4218	46·4	20
182, CH	Female	119·80	2487	41·6	20
173, AR + CH	Male	70·27	877	42·1	33
176, AR+CH	Male	85·27	1072	38·4	33
175, AR	Male	60·82	806	46·7	33
177, CH	Male	65·09	1284	55·1	33
181, AR+CH	Male	138·90	2523	36·2	20
180, AR	Male	90·65	2082	50·4	20
182, CH	Male	82·45	1736	50·5	20

comparisons of the polymorphic and monomorphic populations sampled simultaneously. The polymorphic populations Nos. 173 and 176 produced respectively $99{\cdot}9\pm7{\cdot}1$ and $131{\cdot}5\pm8{\cdot}4$ females and $70{\cdot}3\pm5{\cdot}2$ and $85{\cdot}3\pm5{\cdot}8$ males per cup. The differences between Nos. 173 and 176 are again ascribable to the environment (see above). The monomorphic populations, Nos. 175 and 177, yielded respectively $86{\cdot}2\pm7{\cdot}9$ and $97{\cdot}9\pm9{\cdot}3$ females and $60{\cdot}8\pm5{\cdot}0$ and $65{\cdot}1\pm6{\cdot}3$ males. The productivity of the monomorphic populations is thus not significantly lower than No. 176, both for females and for males. Since the populations Nos. 175, 176, 177 were kept in identical environments, they are fully comparable, while No. 173 is not quite so.

In the second series, the polymorphic population No. 181 produced $188{\cdot}3\pm13{\cdot}3$ females and $138{\cdot}9\pm11{\cdot}5$ males per cup. The monomorphic populations Nos. 180 and 182 yielded only $139{\cdot}8\pm14{\cdot}9$ and $119{\cdot}8\pm11{\cdot}4$ females and $90{\cdot}7\pm10{\cdot}5$ and $82{\cdot}5\pm9{\cdot}6$ males per cup. The productivity of the polymorphic population is again significantly greater.

Table 2 also shows the variances and the coefficients of variation of the productivity in the different populations. The productivity of the polymorphic populations is consistently less variable than that of the monomorphic ones. The six variation coefficients for the polymorphic populations range from 30·8 to 42·1, and for the monomorphic ones from 41·6 to 53·9. The first series of populations

TABLE 3

Weight (in mg.) of flies in polymorphic populations

No. 173			No. 176			No. 181		
Mean	s^2	Flies	Mean	s^2	Flies	Mean	s^2	Flies
Females			Females			Females		
0·804	0·0120	80	0·889	0·0291	110	0·912	0·0140	127
0·897	0·0234	65	0·845	0·0206	160	0·786	0·0223	164
0·783	0·0116	83	0·949	0·0153	117	0·737	0·0150	143
0·738	0·0112	96	0·910	0·0133	115	0·873	0·0153	132
0·845	0·0177	100	0·659	0·0161	134	0·816	0·0228	247
0·929	0·0199	91	0·776	0·0209	143	0·730	0·0354	261
0·975	0·0298	93	0·754	0·0291	87	0·678	0·0218	265
1·026	0·0133	99	0·752	0·0158	148	0·691	0·0293	204
0·765	0·0309	215	0·688	0·0247	226	1·108	0·0245	162
0·727	0·0201	140	0·741	0·0300	150	1·037	0·0148	125
Males			Males			Males		
0·721	0·0198	39	0·769	0·0119	53	0·803	0·0138	59
0·796	0·0131	45	0·729	0·0099	90	0·665	0·0211	124
0·686	0·0084	34	0·789	0·0101	77	0·660	0·0145	92
0·636	0·0104	79	0·770	0·0054	41	0·762	0·0096	70
0·679	0·0148	70	0·601	0·0100	69	0·709	0·0157	145
0·846	0·0103	59	0·700	0·0117	111	0·635	0·0253	217
0·803	0·0145	68	0·696	0·0150	57	0·656	0·0159	197
0·866	0·0069	55	0·687	0·0140	84	0·633	0·0142	157
0·674	0·0206	139	0·604	0·0138	169	1·041	0·0165	124
0·665	0·0120	115	0·673	0·0141	145	0·892	0·0137	84

seems to be more variable than the second, but the difference is not significant.

Note should be taken of the deviation from the normal ratio of sexes observed regularly in all populations. The male sex is deficient, forming only 39 per cent. to 41 per cent. of the total. Such a scarcity of males is usual in overpopulated cultures, the male being obviously the weaker sex.

(iii) *Weight of the flies*

The mean weights of the female and male flies in the different populations are reported in tables 3 and 4. For each population all the flies hatching in each of ten cups were weighed individually, as stated above in the section on Material and Techniques. The

number of flies in each cup, the mean weights, and the variances of the individual weights (s^2) are given. The data are further summarised in table 5, which shows the unweighted averages of the mean weights of the flies from each of the ten cups in each population. Table 5 shows also the variance of the individual weights of the flies and the variances of the mean weights in the different cups. The variances of "individuals" are unweighted means of the variances reported in

TABLE 4

Weight (in mg.) of flies in monomorphic populations

No. 175—AR			No. 177—CH			No. 180—AR			No. 182—CH		
Mean	s^2	Flies	Mean	s^2	Flies	Mean	s^2	Flies	Mean	s^2	Flies
Females			Females			Females			Females		
0·786	0·0571	141	0·894	0·0222	70	0·932	0·0331	98	0·837	0·0269	150
0·940	0·0325	141	0·669	0·0176	133	0·915	0·0292	81	0·875	0·0315	100
0·808	0·0278	53	0·786	0·0176	71	1·047	0·0312	58	0·719	0·0248	174
0·954	0·0382	59	0·892	0·0171	76	0·858	0·0218	47	0·762	0·0161	1:1
0·760	0·0332	99	0·848	0·0334	119	0·706	0·0173	180	0·564	0·0175	134
0·962	0·0324	36	0·852	0·0261	103	0·773	0·0330	258	0·806	0·0282	150
0·762	0·0279	57	0·729	0·0422	64	0·754	0·0271	276	0·874	0·0184	150
0·892	0·0319	119	1·023	0·0317	91	0·925	0·0489	176	1·032	0·0419	45
0·680	0·0348	134	0·726	0·0262	213	1·060	0·0240	84	1·072	0·0124	54
0·742	0·0155	187	0·653	0·0207	331	1·071	0·0125	116	1·085	0·0222	42
Males			Males			Males			Males		
0·682	0·0317	104	0·720	0·0169	53	0·761	0·0196	53	0·728	0·0175	128
0·760	0·0207	86	0·618	0·0142	78	0·876	0·0243	57	0·699	0·0235	81
0·641	0·0175	35	0·738	0·0152	49	0·912	0·0119	36	0·634	0·0157	91
0·773	0·0146	34	0·750	0·0150	30	0·756	0·0141	30	0·649	0·0081	60
0·696	0·0192	83	0·746	0·0122	71	0·703	0·0208	184	0·674	0·0192	96
0·762	0·0154	24	0·759	0·0171	79	0·641	0·0154	97	0·533	0·0063	85
0·724	0·0160	36	0·634	0·0197	44	0·665	0·0142	181	0·768	0·0124	83
0·779	0·0153	66	0·905	0·0237	87	0·826	0·0302	112	0·811	0·0218	15
0·581	0·0178	90	0·611	0·0144	136	0·904	0·0168	65	0·890	0·0106	33
0·650	0·0136	145	0·551	0·0144	212	0·889	0·0126	49	0·919	0·0132	27

tables 3 and 4; these are, then, the average variances of the individual fly weights emerging in the same cup. The variances of "means" are computed from the ten average weights shown, for each population and sex, in tables 3 and 4; these variances characterise the reactions of the flies to the differences in the environmental conditions found in the different cups.

Flies from the polymorphic populations are neither consistently heavier nor lighter than those from the monomorphic ones. Examination of the tables and of fig. 1 shows, however, that there exists, as expected, a rather weak but significant negative correlation between the numbers of the flies emerging per cup and their weights. This only

means that when fewer flies develop in a given amount of food they are bigger flies. The heterosis in polymorphic populations does not make the flies large. Average weights of female flies exceeding 1 mg. have been observed only among samples taken during spring and summer of 1958, both in monomorphic and in polymorphic populations. Examination of fig. 1 suggests, however, that, with equal population

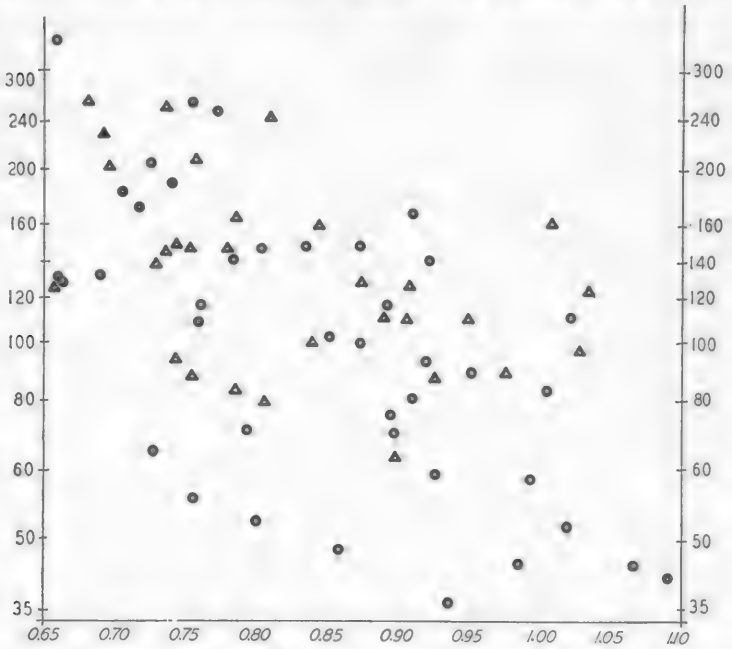

FIG. 1.—Mean weight (in mg.) of a female (abscissæ) plotted against the log. of the number of flies hatching in a cup (ordinates).
Circles—monomorphic populations. Triangles—polymorphic populations.

densities, the flies tend to be slightly heavier in polymorphic than in monomorphic populations.

The data on the variability of the body weight are more informative. Table 5 reports the variances of the weights of individuals emerging from the same food cup; consider these data separately for the two sexes and for the two series of the experimental populations (Nos. 173-177 and Nos. 180-182). With the sole exception of males in population 182, the polymorphic populations show smaller variances than the comparable monomorphic ones. To eliminate the possible disturbing effects of the differences in the average weights, we have computed the coefficients of variation for individuals emerging from the same cup. Females are more variable in weight than males; the variation coefficients range from 16·2 to 18·4 in polymorphic,

and 18·0 to 21·7 in monomorphic populations for females, from 15·0 to 17·0 in polymorphic and 16·7 to 19·1 in monomorphic populations for males. No consistent trend is shown by the variances of the mean weights; it may, or may not, be significant that in three of the four possible comparisons the monomorphic CH populations are the most variable ones.

TABLE 5

Mean weight (in mg.) of a fly, and variances of individual (intra-cup) and of mean (inter-cup) weights in polymorphic and monomorphic populations

Population	Sex	Mean	Variance of individuals	Variance of means
173, AR+CH	Female	0·8485	0·01897	0·0107
176, AR+CH	Female	0·7963	0·02148	0·0094
175, AR	Female	0·8286	0·03312	0·0101
177, CH	Female	0·8072	0·02415	0·0134
181, AR+CH	Female	0·8368	0·02151	0·0213
180, AR	Female	0·9039	0·02780	0·0173
182, CH	Female	0·8626	0·02399	0·0273
173, AR+CH	Male	0·7371	0·01217	0·0069
176, AR+CH	Male	0·7018	0·01159	0·0043
175, AR	Male	0·7047	0·01817	0·0044
177, CH	Male	0·7031	0·01626	0·0103
181, AR+CH	Male	0·7456	0·01603	0·0178
180, AR	Male	0·7842	0·01799	0·0096
182, CH	Male	0·7305	0·01483	0·0142

(iv) *Biomass*

Multiplying the number of the flies hatching in the different cups by their mean weights, we obtain the total weights of the flies emerging in the different populations per unit of food (the nutrient medium in the cup). The figures so obtained are shown in table 6. The results

TABLE 6

Total weights (biomass) in mg. produced per cup in different populations

Sex	Polymorphic populations			Monomorphic populations			
	No. 173	No. 176	No. 181	No. 175	No. 177	No. 180	No. 182
♀	84·82	104·71	157·61	72·17	78·15	126·32	103·33
♂	51·80	59·87	103·52	42·86	45·76	63·88	60·23
Total	136·62	164·58	261·13	115·03	123·91	190·20	163·56

are clear and consistent if comparable populations are compared. Consider the Nos. 173-177 series first. In the polymorphic populations, a cup produced from 85 to 105 mg. of females and from 52 to 60 mg. of males, *i.e.* from 137 to 165 mg. of flies. In the monomorphic populations a cup yielded from 72 to 78 mg. of females and from 43 to 46 mg.

of males, *i.e.* from 115 to 124 mg. of flies. In the Nos. 180-182 series, the polymorphic population yielded 158 mg. of females and 104 mg. of males, *i.e.* about 261 mg. of flies per cup. The comparable monomorphic populations yielded 103 to 126 mg. of females and 60 to 64 mg. of males, *i.e.* 164 to 190 mg. of flies. The polymorphic populations are clearly more efficient producers of the living substance of the species *Drosophila pseudoobscura*.

(v) *Wing length*

The mean wing lengths characterising the different populations are shown in table 7 together with the variances attached to these

TABLE 7

Mean wing length (in mm.) and intra-cup (individual) variance and variance of means (inter-cup) in different populations studied

Population	Sex	Mean	Intra-cup variance	Inter-cup variance
173, AR+CH	Female	2·225	0·01169	0·00268
176, AR+CH	Female	2·236	0·00915	0·00207
175, AR	Female	2·252	0·01674	0·00452
177, CH	Female	2·197	0·01833	0·00419
181, AR+CH	Female	2·249	0·01016	0·00126
180, AR	Female	2·157	0·01099	0·00277
182, CH	Female	2·156	0·01217	0·00335
173, AR+CH	Male	2·020	0·00846	0·00216
176, AR+CH	Male	2·041	0·00622	0·00153
175, AR	Male	2·039	0·00945	0·00243
177, CH	Male	2·013	0·00703	0·00212
181, AR+CH	Male	2·062	0·00739	0·00093
180, AR	Male	2·034	0·01099	0·00377
182, CH	Male	1·969	0·01296	0·00139

values. In the 170 series of populations it seems that the AR flies from population 175 have the longest wings and CH flies from population 177 have the shortest wings, the flies from the polymorphic populations being of intermediate wing length. In the 180 series on the other hand, the mean wing length in the polymorphic population is greater than in both of the monomorphic populations. There is thus an indication that polymorphic populations *may* produce larger individuals than monomorphic populations but this is not inevitable and presumably depends largely upon external factors and upon the relative numbers of individuals produced in the two types of populations. It is to be expected that where more individuals are produced in a culture these will be smaller than when there are fewer. In fact the polymorphic populations produce more flies whose mean wing length is usually as great as, or greater than, those from monomorphic populations.

Comparison of the variances of wing length in the different populations gives, with a single exception, a quite consistent picture, namely that the polymorphic populations are less variable than the

monomorphic ones. Let us consider the intra-cup (individual) variances first. In the 170 series of populations, the females in polymorphic populations show variances of 0·0117 and 0·0092, and in monomorphic ones 0·0167 and 0·0183; the males in polymorphic populations, 0·0085 and 0·0062, and monomorphic ones, 0·0094 and 0·0070 (the population No. 177 being exceptional). In the 180 series, the females in the polymorphic population have the variance of 0·0102 and in the monomorphic ones, 0·0110 and 0·0122; the males show 0·0074 in the polymorphic and 0·0110 and 0·0130 in the monomorphic ones. The variances of means (intra-cup) behave similarly. In the 170 series, the females of the polymorphic populations give 0·0027 and 0·0021, and of the monomorphic ones 0·0045 and 0·0042; the males in the polymorphic, 0·0015 and 0·0022, and in the monomorphic ones, 0·0024 and 0·0021 (the population No. 177 is again exceptional). In the 180 series, the polymorphic population gives 0·0013 and the monomorphic ones, 0·0028 and 0·0033 for females; and for males, 0·0009 in the polymorphic and 0·0038 and 0·0014 in the monomorphic populations.

The inescapable conclusion is that populations segregating for large genetic units are in general showing less phenotypic variation than populations pure breeding for these same units of inheritance.

(vi) *Asymmetry*

The mean intra-fly difference between right and left wings, *i.e.* wing asymmetry, calculated for each population is shown in table 8.

TABLE 8

Mean asymmetry of wings in microns in flies in different populations

Sex	Polymorphic populations			Monomorphic populations			
	No. 173	No. 176	No. 181	No. 175	No. 177	No. 180	No. 182
♀	8·287	8·999	8·906	10·174	10·548	10·153	8·916
♂	7·913	8·459	7·971	9·831	9·659	9·259	8·542

It can readily be seen that, restricting comparisons to within the 170 and 180 groups of populations respectively, flies from monomorphic populations are characterised by higher average values of wing asymmetry than flies from polymorphic populations. In fact, an analysis of variance shows that the apparent differences are statistically highly significant (table 9) in all except one of the informative comparisons (181 < 182). In this connection it is worth noting that the flies from population 182 are here considerably shorter in wing length than those from 181 (see table 7 for mean wing lengths) and the asymmetry value is therefore correspondingly lower than it would have been had the mean wing length of flies from 182 been more

nearly equal to that of the 181 flies. This is because the amount of asymmetry of bilaterally paired organs or structures appears to depend partly upon the absolute size of the organs themselves (Thoday, 1958). Thus, all other things being equal, flies with shorter wings have more symmetrical wings than flies with longer wings. In this case, population 182 flies have significantly shorter wings than population 181 flies but the same level of quantitative asymmetry; it seems reasonable to suppose that the homeostatic mechanisms of development are less efficient in the 182 flies than in 181 flies. This is, then, in general

TABLE 9

Comparisons of mean values of wing asymmetry in polymorphic and monomorphic populations

Females			Males		
Polym.	Monom.	P	Polym.	Monom.	P
173	<175	<0·001	173	<175	<0·001
173	<177	<0·001	173	<177	<0·001
176	<175	<0·001	176	<175	<0·001
176	<177	<0·001	176	<177	<0·001
181	<180	<0·01	181	<180	<0·01
181	<182	<0·05	181	<182	<0·05

agreement with the conclusion to be drawn from the other comparisons in table 9, that is, that in the polymorphic populations development is on average more homeostatic or better canalised than in the monomorphic populations.

4. DISCUSSION

Among the seven experimental populations which we have studied, four were monomorphic and three were polymorphic. Among the monomorphic ones, two were homozygous for third chromosomes with the AR gene arrangement and two homozygous for the CH arrangement. The polymorphic populations contained both AR and CH chromosomes, their frequencies approaching equilibrium values, *i.e.* AR being about twice as frequent as CH. Among the zygotes formed in the polymorphic populations, slightly fewer than half were the heterokaryotype AR/CH, about one-third were the homozygotes AR/AR, and the remainder belonged to the homokaryotype CH/CH. Slightly more than half of the flies in the polymorphic population were, therefore, chromosomally identical with the flies in the monomorphic populations AR/AR and CH/CH, respectively.

The findings described on the preceding pages must be interpreted in the light of the known composition of the experimental populations. We have found that the polymorphic populations differ in a number of

respects from the monomorphic ones. The simplest explanation of this would seem to be that the properties of the polymorphic populations which distinguish them from the monomorphic ones are due to the presence in the former of the AR/CH karyotype. The greater numbers of flies and the greater biomass produced in the polymorphic populations may, thus, be due to greatly superior survival rates of the AR/CH class compared to the homozygotes. It is less easy to interpret similarly the lower variances found in the polymorphic populations, for this would mean that the AR/CH heterozygotes show a quite remarkably low variability. After all, the genetic component of the variance should be, if anything, greater in the polymorphic populations. Consideration should therefore be given to a possible alternative. Levene, Pavlovsky and Dobzhansky (1954, 1958) found that the adaptive values of the karyotypes in experimental populations may be quite appreciably modified by the presence or absence of other karyotypes in the same medium. The fitness and other properties of, for example, AR/AR may, then, not be the same in a pure culture and in a culture in which AR/AR shares the environment with AR/CH and with CH/CH. There may quite conceivably exist a kind of mutual facilitation between the karyotypes which normally live side by side in natural populations. AR/AR and CH/CH may actually benefit by living in the vicinity of each other and of the AR/CH karyotype.

Regardless of whether the differences between the polymorphic and the monomorphic populations are due to a superiority of the heterozygotes or to mutual facilitation, it is important that these differences are of a kind which might be expected to confer a greater adaptedness on the former. The ability to produce a greater number of individuals and a greater biomass from a given amount of food would certainly be advantageous under most natural conditions. The lack of heterosis expressed in a greater weight or a greater size of the flies in the polymorphic populations is certainly compatible with the above view. In the first place, when a culture with a fixed amount of nutrients produces more flies these may well be smaller flies. Secondly, examination of fig. 1 suggests that there is a tendency for the flies from polymorphic populations to be heavier than flies from monomorphic ones when they develop in cultures with similar degrees of crowding (the triangle symbols in fig. 1 tend to be grouped above and to the right of the circles). And thirdly, it appears that flies from crowded cultures are as fecund as those from uncrowded ones if they are treated equally as adults (Spiess, 1958, see, however, Saveliev, 1928; this matter needs reinvestigation). Granted the unsatisfactory state of our understanding of Drosophila ecology, it would seem that a population which produces more flies is likely to have an advantage over one producing larger flies.

It is less easy to evaluate the adaptive significance of the lower variances of the productivity, of the weights of the individuals developing in the same cup, and of the lower degree of asymmetry of the

wing length observed in the polymorphic populations. Dobzhansky and Wallace (1953), Dobzhansky, Pavlovsky, Spassky, and Spassky (1955), and Dobzhansky and Levene (1955) all found that the survival rates of heterozygous genotypes in Drosophila are less variable than those of the homozygotes. Furthermore, among the homozygotes, those less viable over a range of environments tend to be most variable (this is borne out also by unpublished data of Dobzhansky and Spassky). Interpretation of these findings has not been free from confusion; some writers alleged that other writers believed that the low variability of the survival rates caused these rates to be high. Perhaps the following comment may be helpful. Some genotypes endow their carriers with a superior developmental homeostasis, or, to use Waddington's (1957) terms, superior canalisation or superior homeorhesis, in a given range of environments. Other genotypes yield a less versatile developmental homeostasis. A superior homeostatic buffering should result in preservation of life and successful development, and consequently in a high viability, in a variable environment. But the same superior homeostatic capacity should make the survival rates over a certain range of environments more uniform, *i.e.* less variable. Uniformity of the survival rates is neither the cause nor the consequence of high viability; high survival and uniform survival are both conditioned by superior homeostasis (or canalisation, or homeorhesis).

Mather (1953) and Tebb and Thoday (1954) found that the numbers of sternopleural bristles on the two sides of the body in *Drosophila melanogaster* are more often asymmetrical in homozygotes than in heterozygotes. Although Thoday (1958) has shown that genetic imbalance rather than homozygosity as such is responsible for greater asymmetry in some cases, this agrees with our finding that the two wings of a fly are more often asymmetrical (different in length) in monomorphic than in polymorphic populations. Waddington (1957) ascribes asymmetries to "developmental noise", and draws a distinction between this phenomenon and the developmental instability resulting from imperfect homeostatic buffering. We feel that these phenomena are likely to be rather closely related; anyway, it is hardly an accident that heterozygous genotypes, at least those heterozygous for coadapted gene complexes derived from the same population, show both low variance in traits concerned with fitness and low asymmetry (see also Lerner, 1954).

We conclude that experimental populations of *Drosophila pseudoobscura* polymorphic for AR and CH chromosomes are superior in fitness to populations monomorphic for AR or for CH. This assuredly does not mean that monomorphic populations of this kind cannot be maintained; at least under laboratory conditions they are reasonably prosperous. But as Li (1955) has justly remarked: "Should man become a one-legged animal, we cannot be sure that mankind will perish from earth when raised in pure cultures", *i.e.* if all men should

be one-legged animals. Polymorphic populations in Drosophila are superior to monomorphic ones in the same sense in which populations of two-legged men are superior to one-legged ones.

5. SUMMARY

Seven experimental populations of *Drosophila pseudoobscura* have been studied in laboratory population cages. Three of them were polymorphic for the AR and CH gene arrangements in the third chromosomes, two were monomorphic for AR, and two for CH chromosomes. The polymorphism is balanced owing to heterosis in the AR/CH heterozygotes. After the polymorphic populations had approached the equilibrium frequencies of AR and CH, samples were taken from all the populations. The samples consisted of cups with the nutrient medium, withdrawn from the cages at a rate of, at most, one cup per week, during the period of mass pupation of the larvæ in the cups. The following data were obtained from the samples: (1) numbers of female and male flies hatching, (2) weights of individual flies, (3) length of the wings, (4) asymmetry of the lengths of the two wings of the same individual, and (5) total weight (biomass) of the flies hatching from a cup.

The results obtained show that (*a*) the polymorphic populations produce more individuals than do the monomorphic ones (*cf.* table 2), (*b*) the numbers of individuals hatching per cup are less variable in the polymorphic than in the monomorphic populations (table 2), (*c*) the mean weights of a fly are about the same in comparable polymorphic and monomorphic populations (tables 3-5), (*d*) the variances of weights of individuals hatching in the same cup are smaller in polymorphic than in monomorphic populations (tables 3-5), (*e*) the biomass produced is greater in polymorphic than in monomorphic populations (table 6), (*f*) the mean length of the wings is about the same in polymorphic and monomorphic populations (table 7), (*g*) the variance in wing length is generally greater in the monomorphic populations (table 7), (*h*) the asymmetry of the wings is greater in monomorphic than in polymorphic populations (tables 8 and 9).

We conclude that the polymorphic populations which we have studied are superior in Darwinian fitness to the monomorphic ones. It is conjectured that this superior fitness may be due not only to high adaptive value of the heterokaryotype AR/CH, but also to favourable interaction (mutual facilitation) between the carriers of the different karyotypes.

6. REFERENCES

DOBZHANSKY, TH. 1948. Genetics of natural populations XVIII. *Genetics*, *33*, 588-602.

DOBZHANSKY, TH. 1957. Mendelian populations as genetic systems. *Cold Spring Harbor Symp. Quant. Biol.*, *22*, 385-393.

DOBZHANSKY, TH., AND LEVENE, H. 1955. Genetics of natural populations. XXIV. *Genetics*, *40*, 797-808.

DOBZHANSKY, TH., PAVLOVSKY, O., SPASSKY, B., AND SPASSKY, N. 1955. Genetics of natural populations. XXIII. *Genetics, 40,* 781-796.

DOBZHANSKY, TH., AND WALLACE, B. 1953. The genetics of homeostasis in Drosophila. *Proc. Nat. Acad. Sci., 39,* 162-171.

LERNER, I. M. 1954. *Genetic Homeostasis.* John Wiley, New York.

LEVENE, H., PAVLOVSKY, O., AND DOBZHANSKY, TH. 1954. Interaction of the adaptive values in polymorphic experimental populations of *Drosophila pseudoobscura. Evolution, 8,* 335-349.

LEVENE, H., PAVLOVSKY, O., DOBZHANSKY, TH. 1958. Dependence of the adaptive values of certain genotypes in *Drosophila pseudoobscura* on the composition of the gene pool. *Evolution, 12,* 18-23.

LEVINE, L. 1955. Genotypic background and heterosis in *Drosophila pseudoobscura. Genetics, 40,* 832-849.

LI, C. C. 1955. The stability of an equilibrium and the average fitness of a population. *Amer. Naturalist, 89,* 281-295.

MATHER, K. 1953. Genetical control of stability in development. *Heredity, 7,* 297-336.

SAVELIEV, V. 1928. On the manifold effect of the gene vestigial in *Drosophila melanogaster. Tr. Soc. Natur. Leningrad, 63,* 65-88.

SPIESS, E. 1958. Chromosomal adaptive polymorphism in *Drosophila persimilis. Evolution, 12,* 234-245.

TEBB, G., AND THODAY, J. M. 1954. Stability in development and relational balance of X-chromosomes in *Drosophila melanogaster. Nature, 174,* 1109.

THODAY, J. M. 1958. Homeostasis in a selection experiment. *Heredity, 12,* 401-415.

WADDINGTON, C. H. 1957. *The Strategy of the genes.* Allen & Unwin, London.

EFFECTS OF DISRUPTIVE SELECTION

III. COUPLING AND REPULSION

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1. INTRODUCTION

It has already been shown experimentally (Thoday, 1958, 1959) that disruptive selection (Mather, 1953) or centrifugal selection (Simpson, 1944) can increase and that stabilising (or centripetal) selection can reduce the genetic flexibility of a population. The effects of disruptive selection can also extend so far as to render a population polymorphic (Thoday and Boam, 1959), as Mather (1955) had expected on general grounds.

These experiments involved selection for sternopleural chæta number in populations initiated from a wild stock of *Drosophila melanogaster*. They were open to interpretation in terms of Mather's (1943) models, on the supposition that disruptive selection can increase the frequency of coupling linkages of genes hitherto present mostly in repulsion complexes, and that stabilising selection can increase the frequency of repulsion complexes and, if populations are small, render them homozygous.

The experiments to be reported here complement those described before. They were initiated from a cross between two lines differing widely in chæta number, so that relevant genes could be assumed to have been in the coupling phase of linkage at the beginning of selection. They also supplement the previous experiments in that the lines crossed differed at four marker loci, two of which were known to be linked to a chromosome region with a strong effect on chaeta number. It was hoped that these markers would provide information about the parental or recombinant chromosomes that disruptive or stabilising selection might favour.

2. TERMINOLOGY

The terminology of selection is becoming confusing, and a restatement of the definitions to be used seems desirable. The terms stabilising and disruptive are used here in the sense defined in the first paper of this series (Thoday, 1959) and are only intended to denote the kind of selection exercised by the experimenter. They are not used to describe the expected consequences of selection. These terms and others, however, are often used in relation to the expected consequences of selection. Waddington (1953), for example, felt it necessary to divide stabilising selection into normalising and stabilising according to

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whether the consequences were a reduction of genetic variance or an increase of developmental stability, and Lerner (1958, fig. 1) has felt it necessary to describe disruptive selection as stabilising, presumably because disruptive selection may sometimes stabilise gene frequencies.

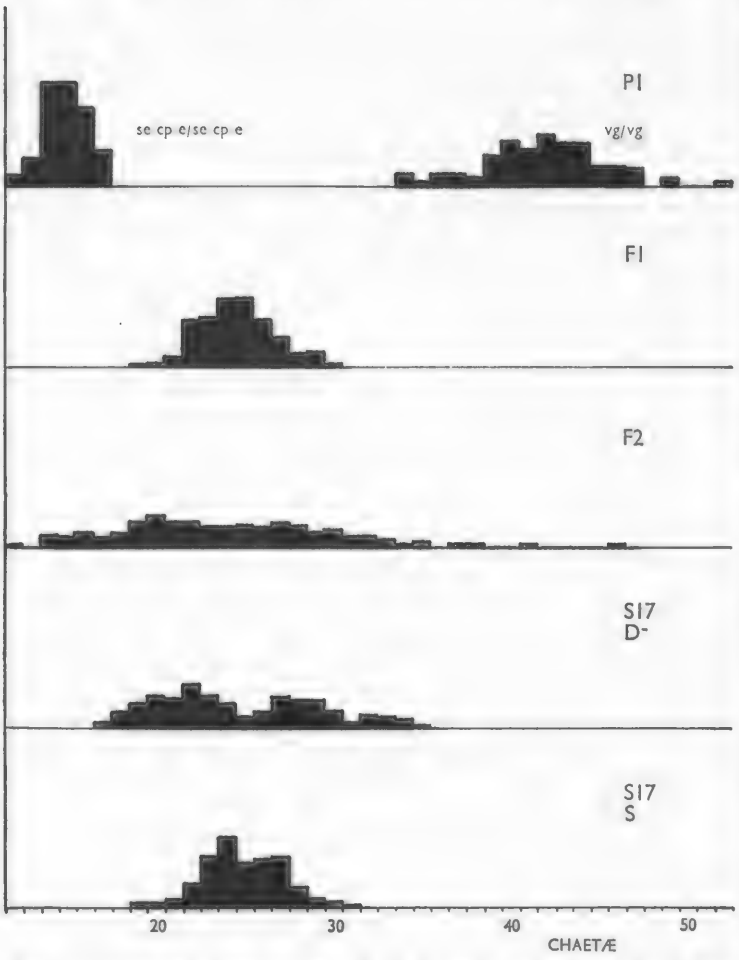

FIG. 1.—Distributions of chæta number in P_1, F_1, F_2 and in the two lines in S17. Each histogram is based on 160 flies except that for F_2 which is based on 320.

In these circumstances it might be better to follow Falconer's (1958) use of Simpson's relatively neutral terms, centripetal and centrifugal selection. But it seems a pity to change terminology in the middle of a series of papers. The terms disruptive and stabilising

will still be used, therefore, but the reader is reminded that they are used in senses independent of their expected consequences. Stabilising selection is selection for the mean. Disruptive selection is selection away from the mean in both directions in the same population.

Waddington (1958) has also suggested that disruptive selection can adequately be regarded as a subtype of directional selection. This is clearly illegitimate as the terms are used here, and is also unsatisfactory when the two do have different consequences.

3. MATERIAL

The experiments have involved the maintenance of two lines, one (S) under stabilising selection, the other (D⁻) under disruptive selection with negative assortative mating. These were both initiated from the same four F_1 single-pair cultures. The F_1 was a cross between a selection line " vg 6 ", whose history is to be described elsewhere, and a stock homozygous for the third chromosome recessives *se*, *cp* and *e*.

The line vg 6 is homozygous for the second chromosome recessive *vg* and, when the F_1 was made, had a mean sternopleural chaeta-number of 40 as a result of 72

TABLE 1

Mean chaeta-numbers of F_2 flies classified by vg *and* se *phenotype*

	Non-sepia	Sepia	Difference
Vestigial	28·16	19·55	8·61
Non-Vestigial . . .	24·53	17·65	6·88
Difference	3·63	1·90	

generations of selection for high chaeta number. The mean chaeta number of the *se cp e* stock was about 15. The chaeta number frequency distribution curves for the two parent lines, their F_1 and F_2 are given in fig. 1, which illustrates the very large variance available at the start of the experiments.

It is clear from the F_2 data that chaeta number genes were linked both to the *vg* and to the *se* loci. The vestigial winged flies averaged 25·5 chaetæ and the non-vestigial 23·2 chaetæ. The sepia-eyed flies averaged 18·3 chaetæ and the non-sepia 25·3 chaetæ. Chromosome III is the more important, but there is evidence of interaction between the chromosomes which is summarised in table 1. The effect of the high third chromosomes was greater in *vg* homozygotes.

Some test-crosses have been made to analyse the differences affecting chaeta number that distinguish the vg 6 chromosome III from more normal third chromosomes. These were done earlier in the history of vg 6, well before vg 6 was used to set up the present experiments. They indicated that flies heterozygous for the third chromosomes of vg 6 and the *se cp e* stock, differed from those homozygous for the *se cp e* chromosome by about 3 chaetæ, and that the right arm of the chromosomes did not contribute significantly to this difference, which was confined to regions to the left of the locus *p*. Some of the difference, probably the major part, is to the right of *se* but to the left of *cp*. Most of the difference is to the left of *rt*, but some of it *may* be to the right of *rt*. Marked chromosomes suitable for further analysis have yet to be synthesised, and at present the test-crosses have provided no certain information whether the third chromosomes differ at one or several

loci that affect chaeta number. However, the experiments were initiated on the assumption that the relevant region of the vg 6 chromosome III contained a linked group of polygenes as Mather's (1943) ideas would imply. The results clearly agree with this assumption (see below).

4. MAINTENANCE OF THE LINES

Culture technique, assay, and selection were identical with those described for the D⁻ and S lines of Thoday (1959), with the one exception that no preference for more bilaterally symmetrical flies was exercised in selection.

One hundred and sixty flies, 20 of each sex from each of four cultures were assayed in each line in each generation. Each culture was a single pair culture and, provided that the best single pair cultures were successful, selection involved choosing one fly out of each 20.

TABLE 2

Mating and selection system for D⁻ line

Parents of generation	Culture			
	A	B	C	D
	♀ ♂	♀ ♂	♀ ♂	♀ ♂
n	HA×LC	HB×LD	LC×HA	LD×HB
n+1	HA×LD	HB×LC	LC×HB	LD×HA
n+2	HA×LC	HB×LD	LC×HA	LD×HB
n+3	HA×LD	HB×LC	LC×HB	LD×HA
etc.				

The entries designate the parents used to produce the culture in the generation shown in the first column. H indicates the highest, and L the lowest chaeta number fly found in the appropriate culture. A, B, C and D indicate the culture from which the fly was selected.

The D⁻ (Disruptive selection with negative assortative mating) mating and selection system is reproduced in table 2. The S line mating system was identical, but selection in this line was aimed at the F_1 mean value (24·5). The female with the nearest to 25 chaetæ and the male with the nearest to 24 chaetæ were chosen. Selection was started in F_2.

Care had to be taken to ensure that the 20 flies of one sex chosen from a culture for assay were chosen at random with respect to the marker phenotypes segregating in the cultures. To this end the etherised flies were placed in a pile on a card, and the pile was separated into two with a needle. One-half was then collected into a pile again. The process was repeated until a sample of about 20 was obtained. This was then adjusted to precisely 20 by adding the flies lying nearest to the chosen pile or removing those nearest the discarded

pile. Occasional counts of whole cultures indicated that this procedure did not result in selection of significant excess or deficiency of any of the major-gene phenotypes segregating in the cultures.

The experimental work in F_1 and F_2 was carried out by D. S. Hathaway, then visiting Sheffield as a Fulbright Scholar. The experiment was continued thereafter by the author except that Mr T. B.

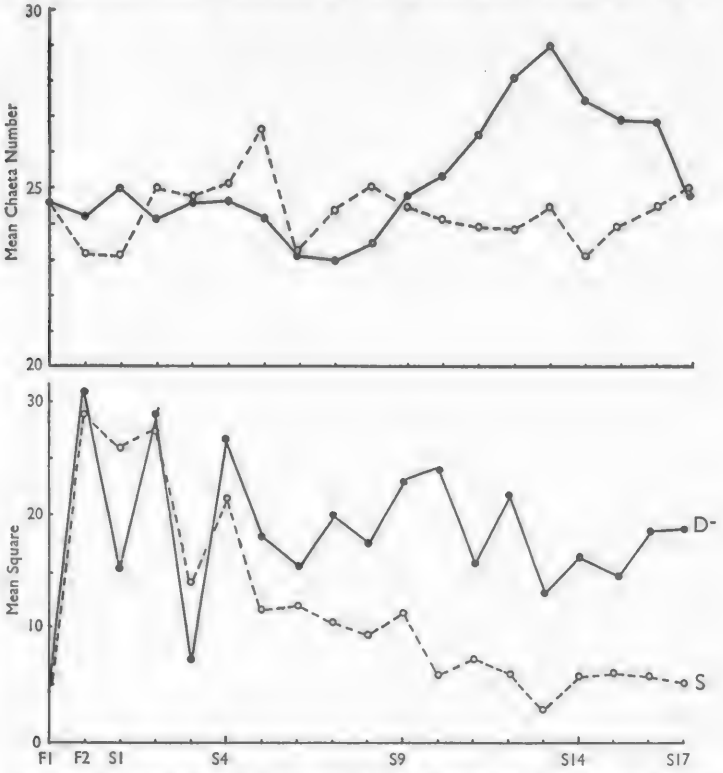

FIG. 2.—Mean chaeta numbers (upper graph) and total variance of chaeta number (lower graph for the two lines. Solid curve D⁻, broken curve S. (N = 159 for each variance estimate.)

Boam maintained the lines during the absence of the author for generations S12, 13 and 14, and did most of the virgin collection.

Serious fertility trouble in the D⁻ line made it necessary to end the experiment after generation S17.

5. RESULTS

(1) *Means and variances*

Fig. 2 illustrates the changes of mean chaeta number in the two lines. The only notable changes occurred in the D⁻ line which for a

period rose to a mean considerably higher than that of the F_1 or the S line, but at the end returned approximately to the F_1 value.

Fig. 2 also illustrates the total variance of each line. The variance in F_2 was very large indeed and was the same in each line. Thereafter the two lines differed.

The S line in F_3 and F_4 (S1 and S2) maintained the high variance of F_2. This was to be expected as selection of intermediates in F_2 must tend to be selection for F_1 genotypes. The variance of the S line fell in S_3, rose again in S_4, but then fell steadily to reach a level similar to that of the F_1.

The D^- line, in the earlier generations of selection fluctuated widely in variance, which was also to be expected. Selection of both extremes in F_2 must tend to select the parental types and negative assortative mating must then reconstitute the F_1 genotype in alternate generations. However, the fluctuations were rapidly damped out so that, from generation S_5 onward, the variance of D^- became comparatively steady, and clearly greater than that of the S line. The final level is midway between the variances of F_1 and F_2.

(ii) *The fate of the marker genes*

(a) *The S line.* The frequencies of homozygotes for *vg*, *cp* and *e* fell rapidly in the S line, but the frequency of *se* homozygotes rose until, in generation S12, all the flies selected were *se* homozygotes. *vg*, *cp* and *e* did not disappear completely. *cp* homozygotes occurred in one culture of S17, *e* homozygotes occurred in one culture of S16, and one culture segregated *vg* homozygotes in S14 though these had not been observed since S_3. The chaeta numbers of these few segregants show clearly that *cp* was still linked to low chaeta number genes and *vg* to high, and it therefore seems likely that stabilising selection could go further, and that in doing so it might eliminate these genes completely.

Fig. 3 illustrates the relative frequencies of the sepia, clipped, sepia-clipped and ++ phenotypes amongst the non-vestigial flies of the line (clipped is hypostatic to vestigial) as far as S10, after which the line was almost homozygous *se*. The figure shows that the initial coupling relationship of *se* and *cp* was rapidly replaced in this line by corresponding repulsion products. At first these were more often +*cp* than *se*+, but later the frequency of *se*+ increased greatly. Despite the few *cp* genes that remain in the line, it is clear that stabilising selection established the *se*+ recombination type of chromosome as typical. Thus the line, while attaining a stable mean and relatively low variance, established in high frequency a repulsion product of the key marker genes originally put in in coupling.

(b) *The D^- line.* The fate of the marker genes in the D^- line has been very different. The line became homozygous *vg* in generation

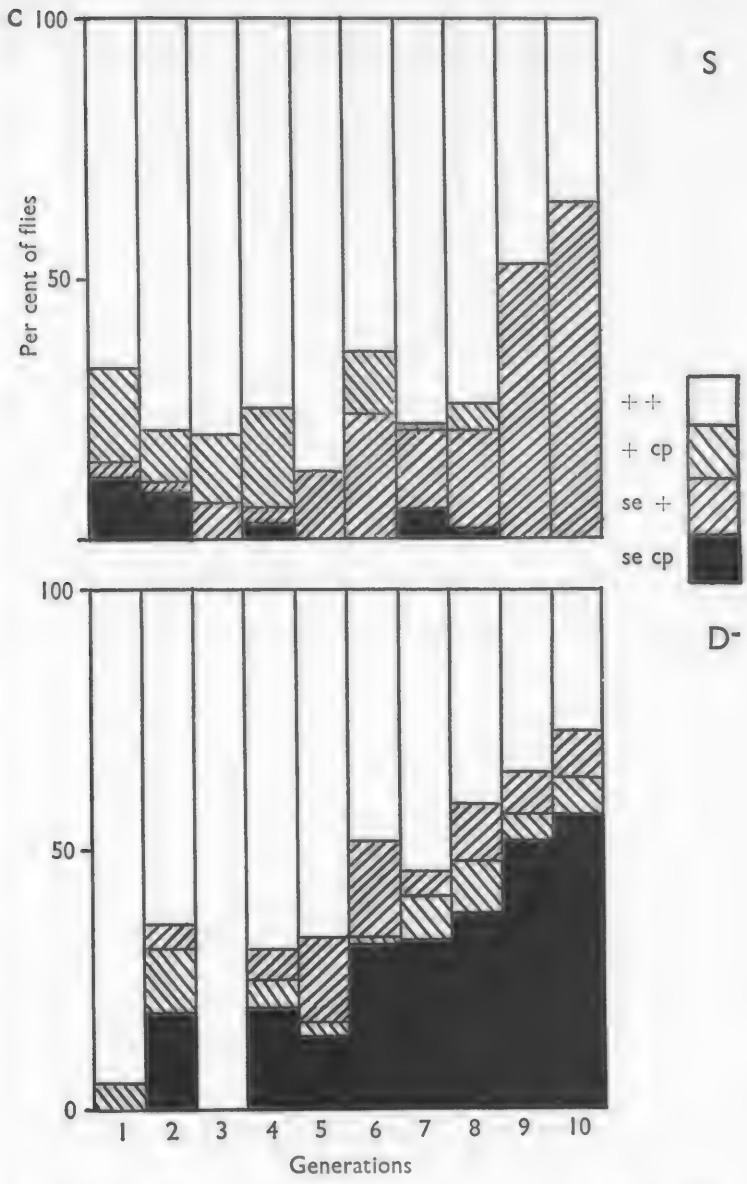

FIG. 3.—Coupling and repulsion *phenotypes* for the markers *se* and *cp* in the two lines: generations S1 to S10. Black, *se cp* homozygotes: Hatched + *cp*/+*cp* or + *cp*/*se cp*, and *se* +/*se*+ or *se* +/*se cp*: white, "wild-type".

15, but maintained the markers *se* and *cp* and their wild-type alleles in high frequency. *cp* gives rise to difficulty as it is hypostatic to *vg* and thus its presence could not be assessed without progeny tests after S10, when the line reached a high *vg* frequency. It was, however, clear from the segregating phenotypes that D⁻ selection favoured *vg*, *se* and *cp* for the frequency of all their homozygotes rose strikingly from generation S3 to S8, the period in which the line was establishing the relatively stable variance roughly midway between that of F_1 and that of F_2, which it maintained thereafter.

Fig. 3 demonstrates the degree to which *se* and *cp* were in coupling linkage in this line. Selection increased the frequency of *se cp* homozygotes which became much more common than in the S line. It seems clear that disruptive selection tended to preserve the coupling linkage of these markers and their wild-type alleles, and in this sense its effects were the opposite of those of stabilising selection.

Because selection in the D⁻ line picked high frequencies of *se cp* homozygotes as low, and of *vg* homozygotes as high parents in each generation, many of the cultures provided test-crosses that made it possible to determine the genotypes of their parents rather completely. Until the line became largely homozygous *vg*, not only the marker genes the parents carried but also their linkage relationships could usually be determined. This was done without using the frequency relationships provided by the data which, since the samples assayed are rather small, are necessarily subject to considerable error. The conclusions concerning the locus *cp* in the later generations have been supplemented by the results of a progeny test that was carried out on the flies available in S15.

The results of this analysis are listed in table 3 which goes a long way toward explaining the history of the line. Flies homozygous for *vg* were often selected as high parents from the beginning, but the low parents also included *vg* homozygotes as early as F_2. Such selection occurred repeatedly, and was responsible for the ultimate *vg* homozygosity of the line.

Most of the third chromosomes selected in F_2 were parental homozygotes except with respect to the *e* locus, which is not closely linked to the known chaeta number affecting region. The flies selected in S1 are all of the F_1 type with respect to *se* and *cp* and those selected in S2 are parental types. In S3 F_1 types were again selected. In S4 some recombinant chromosomes were chosen, but these were not maintained. Though *se*+ and *cp*+ chromosomes recurred, the tendency to pick up ++ chromosomes in the high flies and *se cp* chromosomes in the low continued. Gradually, however, ++ homozygotes disappeared, so that the high flies selected were usually ++/*se cp* and the low were *se cp* homozygotes. There can be little doubt that the disruptive selection was effective in maintaining the loci *se* and *cp* both heterozygous and in the coupling linkage arrangement that they had at the start of the experiment. At the end the line was maintained mainly

TABLE 3

Genotypes of the flies selected in each generation in D-

Female first. v = *vg* s = *se* c = *cp* c = *e*

Selected in generation	Culture			
	A High × Low	B High × Low	C Low × High	D Low × High
F_2(SO)	$\frac{v}{v}\frac{+++}{+++} \times \frac{+}{+}\frac{s\ c\ e}{s\ c\ e}$	$\frac{+}{v}\frac{+++}{s\ \ e} \times \frac{v}{v}\frac{s\ \ e}{s\ c\ e}$	$\frac{+}{v}\frac{s\ c\ e}{s\ c\ e} \times \frac{+}{v}\frac{++e}{+++}$	$\frac{+}{+}\frac{s\ c\ e}{s\ c\ e} \times \frac{v}{v}\frac{++e}{+++}$
S1	$\frac{+}{v}\frac{s\ c\ e}{+++} \times \frac{+}{+}\frac{s\ c\ e}{++e}$	$\frac{v}{v}\frac{+++}{s\ c\ e} \times \frac{+}{v}\frac{s\ c\ e}{++e}$	$\frac{+}{v}\frac{+++}{s\ c\ e} \times \frac{+}{v}\frac{+++}{s\ c\ e}$	$\frac{+}{v}\frac{+++}{s\ c\ e} \times \frac{v}{v}\frac{s\ c\ e}{s+e}$
S2	$\frac{+}{v}\frac{++e}{+++} \times \frac{+}{v}\frac{s\ c+}{s\ c\ e}$	$\frac{v}{v}\frac{++e}{+++} \times \frac{+}{+}\frac{s\ c+}{s\ c\ e}$	$\frac{+}{v}\frac{s\ c+}{s\ c\ e} \times \frac{v}{v}\frac{+++}{++e}$	$\frac{+}{v}\frac{s\ c+}{s\ c\ e} \times \frac{+}{v}\frac{+++}{++e}$
S3	$\frac{v}{v}\frac{+++}{s\ c\ e} \times \frac{+}{v}\frac{+++}{s\ c\ e}$	$\frac{+}{v}\frac{++}{s\ c+} \times \frac{+}{v}\frac{++}{s\ c}$	$\frac{+}{v}\frac{++}{s\ c} \times \frac{v}{v}\frac{++e}{s\ c\ e}$	$\frac{+}{v}\frac{++}{s\ c} \times \frac{+}{v}\frac{++}{s\ c}$
S4	$\frac{+}{v}\frac{+++}{+\ c\ e} \times \frac{+}{v}\frac{s\ c+}{s\ c\ e}$	$\frac{v}{v}\frac{+}{s} \times \frac{v}{v}\frac{s\ c+}{s\ c\ e}$	$\frac{+}{v}\frac{s\ c+}{s\ c\ e} \times \frac{v}{v}\frac{+++}{s\ c+}$	$\frac{+}{v}\frac{s\ c+}{s\ c\ e} \times \frac{v}{v}\frac{+\ c\ e}{s\ c\ e}$
S5	$\frac{v}{v}\frac{++}{s\ c} \times \frac{+}{v}\frac{s\ c+}{s\ c}$	$\frac{v}{v}\frac{+}{s} \times \frac{+}{v}\frac{+\ c\ e}{s\ c\ e}$	$\frac{+}{v}\frac{s\ c+}{s\ c} \times \frac{v}{v}\frac{++}{s\ c}$	$\frac{+}{v}\frac{+\ c\ e}{s\ c\ e} \times \frac{v}{v}\frac{+++}{s\ c+}$
S6	$\frac{v}{v}\frac{+++}{s\ c+} \times \frac{+}{v}\frac{s\ c+}{s\ c\ e}$	$\frac{v}{v}\frac{+}{s} \times \frac{+}{v}\frac{s\ c+}{s\ c\ e}$	$\frac{+}{v}\frac{s\ c+}{s\ c\ e} \times \frac{v}{v}\frac{++e}{s\ c\ e}$	$\frac{+}{v}\frac{+}{s} \times \frac{v}{v}\frac{+}{s\ c}$
S7	$\frac{v}{v}\frac{+++}{s\ c+} \times \frac{+}{v}\frac{s\ c+}{s\ c\ e}$	$\frac{v}{v}\frac{s}{s\ c} \times \frac{+}{v}\frac{+++}{s\ c+}$	$\frac{+}{v}\frac{s\ c+}{s\ c\ e} \times \frac{v}{v}\frac{+++}{s\ c\ e}$	$\frac{+}{v}\frac{+\ c}{s\ c} \times \frac{v}{v}\frac{++}{s\ c}$
S8	$\frac{v}{v}\frac{+++}{s\ c} \times \frac{+}{v}\frac{s\ c+}{s\ c\ e}$	$\frac{v}{v}\frac{+\ \ +}{s\ \ +} \times \frac{+}{v}\frac{s\ c\ e}{s\ c\ e}$	$\frac{v}{v}\frac{s\ c\ e}{s\ c\ e} \times \frac{v}{v}\frac{+++}{s\ \ e}$	$\frac{+}{v}\frac{s\ c+}{s\ c\ e} \times \frac{v}{v}\frac{+++}{s\ c\ e}$
S9	$\frac{v}{v}\frac{++}{s\ c} \times \frac{v}{v}\frac{+++}{s\ c\ e}$	$\frac{v}{v}\frac{s\ +}{s\ c\ e} \times \frac{+}{v}\frac{s\ c\ e}{s\ c\ e}$	$\frac{v}{v}\frac{+++}{s\ c\ e} \times \frac{v}{v}\frac{+\ \ +}{s\ c\ e}$	$\frac{+}{v}\frac{s\ c+}{s\ c\ e} \times \frac{v}{v}\frac{+\ \ +}{s\ c\ e}$
S10	$\frac{v}{v}\frac{+++}{+++} \times \frac{+}{v}\frac{s\ c\ e}{s\ c\ e}$	$\frac{v}{v}\frac{s\ \ e}{s\ c\ e} \times \frac{v}{v}\frac{s\ c\ e}{s\ c\ e}$	$\frac{v}{v}\frac{+\ \ e}{s\ c\ e} \times \frac{v}{v}\frac{s\ \ +}{s\ c\ e}$	$\frac{+}{v}\frac{s\ c\ e}{s\ c\ e} \times \frac{v}{v}\frac{+++}{+++}$
S11	$\frac{v}{v}\frac{+++}{s\ c\ e} \times \frac{v}{v}\frac{s\ \ e}{s\ c\ e}$	$\frac{v}{v}\frac{s\ \ e}{s\ c\ e} \times \frac{+}{v}\frac{+++}{s\ c\ e}$	$\frac{v}{v}\frac{s\ \ e}{s\ c\ e} \times \frac{v}{v}\frac{+++}{s\ c\ e}$	$\frac{+}{v}\frac{+++}{s\ c\ e} \times \frac{v}{v}\frac{s++}{s+e}$
S12	$\frac{v}{v}\frac{+\ \ +}{s\ \ e} \times \frac{v}{v}\frac{+\ \ e}{s+e}$	$\frac{+}{v}\frac{+++}{s\ \ e} \times \frac{v}{v}\frac{s\ \ e}{s\ c\ e}$	$\frac{v}{v}\frac{s\ \ e}{s\ c\ e} \times \frac{v}{v}\frac{+++}{s\ c\ e}$	$\frac{+}{v}\frac{+}{s+} \times \frac{v}{v}\frac{+\ \ +}{s\ \ e}$
S13	$\frac{v}{v}\frac{+}{s} \times \frac{v}{v}\frac{s\ \ e}{s\ c\ e}$	$\frac{v}{v}\frac{+\ \ e}{s\ \ e} \times \frac{+}{v}\frac{+\ \ +}{s\ c\ e}$	$\frac{v}{v}\frac{+++}{s\ c\ e} \times \frac{v}{v}\frac{+\ \ e}{s\ c\ e}$	$\frac{+}{v}\frac{+}{s} \times \frac{v}{v}\frac{+++}{s\ c\ e}$
S14	$\frac{v}{v}\frac{+++}{s+e} \times \frac{v}{v}\frac{s\ c+}{s\ c\ e}$	$\frac{v}{v}\frac{++e}{s\ c\ e} \times \frac{v}{v}\frac{s+e}{s\ c\ e}$	$\frac{v}{v}\frac{s\ c\ e}{s\ c\ e} \times \frac{v}{v}\frac{++e}{s\ c\ e}$	$\frac{v}{v}\frac{+}{s} \times \frac{v}{v}\frac{+++}{s\ c\ e}$
S15	$\frac{v}{v}\frac{++e}{s\ c+} \times \frac{v}{v}\frac{s\ c\ e}{s\ c\ e}$	$\frac{v}{v}\frac{++e}{s+e} \times \frac{v}{v}\frac{s\ c\ e}{s\ c\ e}$	$\frac{v}{v}\frac{s\ c\ e}{s\ c\ e} \times \frac{v}{v}\frac{++e}{s\ c\ e}$	$\frac{v}{v}\frac{s\ c\ e}{s\ c\ e} \times \frac{v}{v}\frac{++e}{s+e}$
S16	$\frac{v}{v}\frac{++e}{s\ c\ e} \times \frac{v}{v}\frac{s+e}{s\ c\ e}$	$\frac{v}{v}\frac{++e}{s\ c\ e} \times \frac{v}{v}\frac{s\ c\ e}{s\ c\ e}$	$\frac{v}{v}\frac{s\ c\ e}{s\ c\ e} \times \frac{v}{v}\frac{++e}{s\ c\ e}$	$\frac{v}{v}\frac{s+e}{s\ c\ e} \times \frac{v}{v}\frac{+\ \ e}{s\ c\ e}$

Note: Blanks indicate that it is either impossible to determine what genes the flies possessed or impossible to determine their linkage arrangements.

by the following test-cross and its reciprocal, though there were some *se*+*e* chromosomes.

$$\text{High } \frac{vg}{vg}\frac{+}{se}\frac{+}{cp}\frac{e}{e} \times \text{Low } \frac{vg}{vg}\frac{se}{se}\frac{cp}{cp}\frac{e}{e}$$

(c) *Recombination in the D⁻ cultures.* In the last few generations the D⁻ line cultures, of which there were four in each generation, fell into two classes. In any generation two of the cultures test-crossed females heterozygous for eye colour; the other two cultures test-crossed heterozygous males. Each culture segregated two classes of

TABLE 4

Means and variances in the D⁻ line, generations S15 to S17

The figure in brackets is the difference between the chaeta numbers of +/*se* and *se*/*se* flies

Generation	Female test-crosses			Male test-crosses		
	Total mean square	Mean chaeta numbers	Mean square within *se* classes	Total mean square	Mean chaeta numbers	Mean square within *se* classes
		+/*se* *se*/*se*			+/*se* *se*/*se*	
S15	8·90	28·61 26·29 (+2·32)	7·64	20·71	29·37 22·01 (+7·36)	8·32
S16	14·19	29·53 25·29 (+4·24)	9·77	23·61	30·09 21·69 (+8·40)	6·02
S17	17·52	27·04 21·24 (+5·80)	9·38	23·26	28·75 20·72 (+8·03)	7·04
Total	13·54	28·39 24·27 (+4·12)	8·93	22·53	29·40 21·47 (+7·93)	7·13

offspring distinguishable as red-eyed and sepia-eyed. As there is no crossing-over in males, comparisons of these two types of culture provide information concerning the genetic content of the chromosome types that segregated in the line. Table 4 lists the relevant data for the three generations in which *vg* was not segregating, that is S15, 16 and 17.

The data in table 4 demonstrate that the chaeta number factors segregating in the line were complexes, not single loci, for the variance of chaeta-number in the progeny of the female test-crosses was less than that in the progeny of male test-crosses ($F = 1{\cdot}66$, $N_1 = N_2 = 237$, $P < 0{\cdot}01$). Such a difference seems only explicable in terms of recombination between coupled chaeta number genes, which, by producing intermediate repulsion products, would reduce the variance of the progeny of the female test-crosses.

The data also demonstrate that a significant proportion, at least, of the chaeta number factors could readily be separated from the

locus *se* by recombination, for the difference of mean chaeta number distinguishing +/*se* from *se*/*se* flies was greater in the male test-crosses than in the female (table 5). There is also evidence ($P \simeq 0{\cdot}05$) that the variance within the *se* classes was greater in the female test-cross than in the male test-cross progeny, as is to be expected if the linkage between se^+ and high and between *se* and low chaeta number factors was readily broken by recombination.

These data therefore demonstrate that disruptive selection as practised in this line maintained coupling linkages of chaeta number genes as well as of the marker genes, despite the fact that these linkages broke down readily in each generation.

Rough linkage data obtained in the progeny test of generations S15 gave 27·39 per cent. recombination between *se* and *cp*, a value significantly higher than the standard 19·5 of Bridges and Brehme

TABLE 5

Analysis of variance of chaeta-number in the D⁻ line, generations S15 to S17

Source	Sum of squares	*n*	Mean square	F	P
Test-crosses types	50·0521	1	50·0521	5·94	<0·02
+/*se* v. *se*/*se*	4047·5399	1	4047·5399	480·19	*v* small
Interaction	536·2485	1	536·2485	63·62	*v* small
Total	4633·8405	3	1211·2802	143·70	*v* small
Generations	490·5167	2	245·2583	29·10	*v* small
Error	3995·3908	474	8·4291	...	...
Total	9119·7480	479			

(1944). (The value for *cp*—*e* was almost identical with the standard map distance.) The recombination pressure against which disruptive selection was effective was therefore considerable.

These *se*—*cp* recombination frequencies provide no evidence that selection of linked complexes of genes such as occurred in the D⁻ line, has tightened the linkage between them. Such an effect is to be expected in appropriate circumstances (Fisher, 1930), but the circumstances in the present experiment were not appropriate. Selection in the D⁻ line has always been within the progeny of single females which cannot in any simple way be selection for change of recombination frequency (see Thoday and Boam, 1956).

5. DISCUSSION

The variance that was available in the two lines at their origin was very large, and such small populations could hardly be expected to retain all of it, by whatever selection system they were maintained. Only 4 pairs of flies were selected in each generation, one out of only

20 flies was selected, and there were 4 heterozygous marker loci. The possibility of random fixation for any of them must be considerable. The F_2 chaeta-number variance was some fifteen times larger than that characteristic of wild stocks of *Drosophila melanogaster*, and it again might well be reduced by random fixation. It would not therefore have been surprising if the results of these experiments had been difficult to interpret.

Nevertheless the results are clear, and are consistent with the view that disruptive and stabilising selection can have very powerful effects not only on gene frequencies, but also on the frequency of coupling and repulsion phases of linkage of appropriate groups of genes as Mather's (1943) models would lead one to expect.

The stabilising selection line, though it only went to fixation at the marker locus *se*, nevertheless did what was to be expected of it on Mather's views. The majority of chromosomes selected were repulsion products of the key markers *se* and *cp* originally put in in coupling. Taken in conjunction with the evidence of recombination of chaeta loci, and of *se* and chaeta loci, it seems that this repulsion product was selected for because it was a repulsion product of the chaeta loci originally put in in coupling. Whether there was good reason for the selection of *se*+ chromosomes rather than +*cp* chromosomes (which appeared more frequently in the earlier stages) or whether this was merely a matter of chance cannot be decided. A larger population might well have maintained both.

The disruptive selection line went to fixation for *vg* and for the supposedly neutral locus *e* (the latter perhaps a matter of chance), but it maintained the coupling linkages *se cp* and ++ with astonishing permanence, considering the frequency of recombination between these loci. The evidence presented demonstrating recombination of the chaeta number genes clearly indicates that this coupling of the markers was maintained because of their association with the chaeta number loci.

The locus *vg* may have become homozygous in this line by chance, but it seems unlikely, for in any normal population at 25° C. *vg* is rapidly eliminated by natural selection for its normal allele. The evidence in table 1 indicates that there were interactions between the second and third chromosomes in F_2, and that the difference due to the third chromosome was most effective in the presence of the second chromosomes of *vg* 6. It is concluded therefore that *vg* was selected for because linked to useful modifiers that enhanced the differences of chaeta number distinguishing the ++/*se cp* and *se cp*/*se cp* flies.

The difference of chaeta number effected by the *se* regions of chromosome III in the male test-crosses of the recent generations (table 4) is of the same order as that in the F_2 flies that were homozygous *vg* (table 1). Bearing in mind that in F_2 some of the non-sepia flies will have been homozygous for the se^+ chromosome, whereas none of them can have been at S16 to 17 in D^-, it seems clear that

the capacity of the *vg* chromosomes from vg 6 to enhance the effects of the se^+ chromosomes on chaeta number was exploited very fully. The D^- line at the end was polymorphic; its polymorphism depended upon a complex switch "gene" in chromosome III and a genetic background functioning to enhance the effectiveness of this switch. The system is a model example of what Fisher (1930) first proposed must occur, and shows that disruptive selection can produce systems such as we expect to find and have found determining sexual dimorphism, incompatibility, mimicry and polymorphism in general.

TABLE 6

Distributions of chaeta number in the two male test-cross cultures of D^-, generation S17

Class of flies	Chaeta numbers				
	17-21	22	23	24	25-35
Culture C					
♀ +/*se* . .	...	...	...	...	11
se/*se* . .	7	1	1	...	...
♂ +/*se* . .	...	1	1	2	6
se/*se* . .	9	1	...	...	...
Culture D					
♀ +/*se* . .	...	...	...	...	9
se/*se* . .	4	3	3	1	...
♂ +/*se* . .	...	...	...	1	9
se/*se* . .	4	3	2	1	...

The entries give the number of flies in each class.

It may have been the same effect of the *vg* chromosome that was responsible for its near loss in the S line, in which its enhancing effects on chromosome III heterogeneity would operate against the selection practised.

In the previous papers of this series it was shown that stabilising selection can reduce variance, that disruptive selection with negative assortative mating can maintain variance, and that disruptive selection with positive assortative mating can increase variance and set up a cryptic polymorphism. The present experiments confirm the reduction of variance by stabilising selection and provide strong evidence that this involves the establishment of repulsion complexes of relevant genes. They also show that the maintenance of variance by disruptive selection with negative assortative mating involves maintenance of

coupling combinations of relevant genes. The D⁻ line shows us further that a polymorphism can be maintained by disruptive selection with negative assortative mating. This time the polymorphism is not cryptic, but is manifest as a discontinuous morphological difference of eye colour, even though this probably depends solely on linkage of the eye colour locus to the chaeta-number genes primarily subject to selection. The line is also polymorphic for the locus *cp*, though cryptically because *cp* is hypostatic to *vg*, and the polymorphism is manifest, though less clearly, in the key character, chaeta-number. Fig. 1 shows the distribution curves for generation S17. The S line distribution is very similar to that for the F_1, and does not overlap either parent distribution. The D⁻ distribution overlaps both parental distributions and is clearly bimodal. This bimodality is strikingly associated with the distribution of the eye colour markers. In fact, within any sex and culture of the two male test-cross cultures of that generation, there is very little overlap of the chaeta-numbers of red-eyed and sepia-eyed flies (table 6). When recombination is prevented, therefore, the polymorphism is almost discontinuous even in chaeta-number alone.

These experiments therefore confirm the results of those published earlier in showing that disruptive selection can be responsible for polymorphism and they extend them in demonstrating that coupling arrangements of linked genes, and background genotypes enhancing the effects of these switching gene complexes are involved. There seems little reason to doubt that similar and more spectacular results must readily occur in nature where much larger populations and longer times are available.

6. SUMMARY

1. One population (S) has been exposed to stabilising selection and another (D⁻) to disruptive selection with negative assortative mating, each for 17 generations.

2. The two populations were established from the F_1 of a high sternopleural chaeta-number line (vg 6) homozygous for *vg*, and a low chaeta-number line homozygous for *se cp* and *e*. It was known that a considerable part of the genetic material responsible for the high chaeta-number of vg 6 was in the *se—cp* region of chromosome III. The F_2 data showed that the effect of these genes was enhanced in the presence of the *vg* chromosome II of vg 6. The variance of chaeta-number in F_2 was very large indeed.

3. Stabilising selection reduced variance greatly. Disruptive selection maintained a very high variance.

4. Stabilising selection reduced the frequency of the *vg* marker on chromosome II to a low level and presumably also the linked enhancers. It established *se cp*⁺ repulsion chromosomes at high frequency.

5. The disruptive selection line became homozygous for the *vg* marker, but remained heterozygous for the markers *se* and *cp*. These

markers and their + alleles were kept in the coupling phase of linkage despite considerable recombination pressure.

6. It could be shown that recombination of chaeta-number genes also was occurring in the D⁻ line, so that disruptive selection must have been eliminating the repulsion products. This was clearly the cause of the maintenance of the markers in coupling.

7. The disruptive selection line was polymorphic for the locus *se*, and cryptically polymorphic for *cp* (which is hypostatic to *vg*) at the end of the experiment. It was also polymorphic for chaeta-number, this polymorphism depending on a switching gene complex in chromosome III with its effect enhanced by the modifiers in chromosome II.

8. The experiments confirm that disruptive selection can maintain a polymorphism, and show that coupling linkages of appropriate genes are involved. They also show that stabilising selection can function by establishing repulsion linkages of genes hitherto in the coupling phase.

7. REFERENCES

BRIDGES, C. S., AND BREHME, K. 1944. The mutants of *Drosophila melanogaster*. Carnegie Institute of Washington Publication 552.

FALCONER, D. S. 1958. Selection for phenotypic intermediates in *Drosophila*. *J. Genet.*, *55*, 551-561.

FISHER, R. A. 1930. *The Genetical Theory of Natural Selection*. Oxford.

LERNER, I. M. 1958. *The Genetic Basis of Selection*. Wiley, New York.

MATHER, K. 1943. Polygenic balance and natural selection. *Biol. Rev.*, *18*, 32-64.

MATHER, K. 1953. The genetical structure of populations. *Symp. Soc. Exp. Biol.*, *7*, 66-95.

MATHER, K. 1955. Polymorphism as an outcome of disruptive selection. *Evolution*, *9*, 52-61.

SIMPSON, G. G. 1944. *Tempo and Mode in Evolution*. Columbia, New York.

THODAY, J. M. 1958. Effects of disruptive selection : the experimental production of a polymorphic population. *Nature*, *181*, 1124-1125.

THODAY, J. M. 1959. Effects of disruptive selection. I. Genetic flexibility. *Heredity*, *13*, 187-203.

THODAY, J. M., AND BOAM, T. B. 1956. A possible effect of the cytoplasm on recombination in *Drosophila melanogaster*. *J. Genet.*, *54*, 456-461.

THODAY, J. M., AND BOAM, T. B. 1959. Effects of disruptive selection. II. Polymorphism and divergence without isolation. *Heredity*, *13*, 205-218.

WADDINGTON, C. H. 1953. Epigenetics and evolution. *Symp. Soc. Exp. Biol.*, *7*, 186-199.

WADDINGTON, C. H. 1958. *The Strategy of the Genes*. Allen & Unwin, London.

THE EFFECTS OF GAMMA-RADIATION ON CROSSES BETWEEN *LOLIUM PERENNE* AND *FESTUCA PRATENSIS*

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1. INTRODUCTION

SEED-INCOMPATIBILITY, which relates to failure of normal seed development in consequence of impaired endosperm development (Valentine, 1953), has been reported in a wide variety of interspecific crosses, especially among the Gramineæ. It is due either to chromosomal or to genic differences between the genomes of the species involved in the cross, and has been shown to be in large measure responsible for the reproductive isolation which exists between the two related grass species *Lolium perenne* ($2n = 14$) and *Festuca pratensis* ($2n = 14$) (Reusch, 1959*b*). Thus, where in crosses *L. perenne* is used as the seed parent, endosperm development is normal until approximately 72 hours after pollination, but thereafter precocious cell formation in the tissue markedly retards further nuclear division. In reciprocal crosses, on the other hand, cell formation does not occur, but mitotic irregularities eventually lead to the complete disintegration of the endosperm. In the latter case none of the seeds is viable, but in the former some of the caryopses may germinate, depending upon the amount of endosperm formed before cell formation.

Although with the evidence available at present, the possibility of cryptic structural differences between the chromosomes of *L. perenne* and *F. pratensis* cannot be ruled out, it appears that evolutionary divergence between these two species has been mainly genic. For example, it has been shown that in F_1 hybrids, pairing of the chromosomes is normal, and further, that the chiasma frequency of the hybrids closely approximates that of the parental species (Peto, 1933). Significant differences in seed-compatibility between various genotypic combinations of the two species have also been demonstrated (Reusch, 1959*a*).

It was therefore considered possible that ionising radiations could induce gene changes which might break down the barrier to hybridisation. The results presented here indicate that, in certain instances at least, ionising radiations did reduce the degree of seed-incompatibility.

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2. MATERIALS AND METHODS

Three genotypes each of *L. perenne* and *F. pratensis*, which differed widely in their interspecific seed-compatibility (Reusch, 1959*a*) were used for the crosses, namely :

L. perenne	*F. pratensis*
1787bA (1)3—L1	41 (35)—F1
1194bA (1)3—L2	324 (15)—F2
Ba4952 (16)—L3	325 (8)—F3

The system of designation is that employed at the Welsh Plant Breeding Station, Aberystwyth, but for convenience the symbols L1, L2 and L3, and F1, F2 and F3 will be used throughout.

As a source of gamma rays Cobalt^{60} was used, and five different doses, all given over a period of one hour, were applied, namely 250r, 500r, 1000r, 2000r, and 3000r.

The inflorescences were treated about 7 days prior to anthesis, care being taken that they were all at approximately the same stage of development.

All units for any particular cross (generally consisting of approximately 300 florets) were emasculated on the same day, and the pollinations were commenced at strictly comparable times so as to eliminate possible environmental effects on endosperm development (Reusch, 1959*c*).

Two criteria of interspecific compatibility were employed, namely :

(*a*) Seed set : all ovaries showing definite signs of development were recorded as having set seed.

(*b*) Seed viability : all caryopses showing any signs of germination, however weak, were recorded as viable. Since the ability of seeds to germinate is dependent on the presence of at least a small amount of endosperm, the germination data provide an estimate of the seed-compatibility of the various genotypic combinations.

It should be noted that the seed-setting results are based on the total number of florets emasculated, whereas the germination data are expressed as a percentage of the total number of caryopses formed. To a certain degree the two estimates are therefore independent, since it is for example possible to obtain a high germination percentage even when seed-set is low.

3. OBSERVATIONS

(a) *The effect of pollen irradiation on compatibility*

(i) *Seed-set.* Results on the effect of pollen irradiation on seed-set are given in table 1. An analysis of the original data showed that except for the crosses F3 × L1 and F3 × L3, overall differences between treatments were in all cases significant. However, only in the case of three combinations, namely L1 × F2, F1 × L2 and F3 × L2, did seed-set improve as a result of irradiation.

A general feature of the effect of irradiation was a reduction in seed-set at doses of 250-500r. This was followed by an increase at 1000-2000r, but further increases in dose again resulted in a decline.

(ii) *Seed-viability.* The effects of pollen irradiation on seed-viability, although similar to those observed for seed-set, showed greater variability (table 1). If, however, one takes into account the direction of the cross and the normal seed-compatibility of the genotypes, the results still seem to fit into a general pattern.

In the *L. perenne*×*F. pratensis* matings (fig. 1), both the decrease in seed-viability at 250-500r and the recovery at 1000-2000r occurred

TABLE 1

The effect of pollen irradiation on seed-set and seed-viability in crosses between different genotypes of L. perenne *and* F. pratensis

Genotype combination	Percentage seed-set and germination at different doses of γ-rays						χ^2(s)
	Control	250r	500r	1000r	2000r	3000r	
L. perenne (♀) × *F. pratensis* (♂)							
L1×F1 Seed-set	77·6	62·8	66·1	59·0	49·0	47·7	65·4 †
Germination	0·0	0·0	0·0	0·0	0·0	0·0	...
L1×F2 Seed-set	61·8	79·2	81·3	76·2	83·2	83·7	45·7 †
Germination	0·0	0·0	0·0	0·0	0·0	0·0	...
L1×F3 Seed-set	74·9	73·1	66·7	79·3	80·9	67·2	22·4 †
Germination	0·0	0·0	0·0	0·0	0·0	0·0	...
L2×F1 Seed-set	81·5	70·5	65·2	81·1	86·3	73·1	46·1 †
Germination	10·8	7·6	8·0	11·9	8·8	6·5	5·1
L2×F3 Seed-set	85·0	80·3	70·8	84·8	82·9	73·2	26·5 †
Germination	17·6	24·1	6·8	15·1	12·0	10·9	26·7 †
L3×F1 Seed-set	76·7	73·7	66·0	83·6	78·6	60·8	44·9 †
Germination	46·3	25·6	6·9	17·3	31·6	20·0	90·7 †
L3×F2 Seed-set	84·4	72·5	58·6	81·1	64·5	49·6	94·4 †
Germination	44·9	40·6	37·9	54·7	38·7	38·1	12·3 *
L3×F3 Seed-set	76·8	76·3	74·7	76·8	67·4	66·0	15·1 †
Germination	54·1	46·7	42·1	62·0	64·3	62·7	31·3 †
F. pratensis (♀) × *L. perenne* (♂)							
F1×L1 Seed-set	71·7	45·6	50·0	61·3	39·8	25·0	174·6 †
Germination	0·9	0·0	2·1	1·8	0·0	14·3	58·0 †
F1×L2 Seed-set	54·8	73·2	56·6	30·7	43·4	48·0	109·5 †
Germination	0·0	13·4	7·1	4·1	6·4	0·0	35·2 †
F2×L3 Seed-set	61·2	51·6	50·5	51·5	57·6	46·9	14·0 *
Germination	0·0	0·0	0·0	0·0	0·0	0·0	...
F3×L1 Seed-set	53·5	52·3	48·7	58·3	54·8	51·4	5·6
Germination	0·0	0·0	0·0	0·0	0·0	0·0	...
F3×L2 Seed-set	43·8	55·6	62·8	61·0	22·3	13·3	220·0 †
Germination	0·0	0·0	6·1	1·3	0·0	0·0	35·6 †
F3×L3 Seed-set	60·3	57·0	50·4	56·2	52·6	50·0	8·8
Germination	0·0	0·0	0·0	0·0	0·0	0·0	...

* P = 0·05-0·01. † P = ≤0·01.

to varying degrees in different crosses: in the normally incompatible combinations, namely L1×F1, L1×F2 and L1×F3, and in the cross

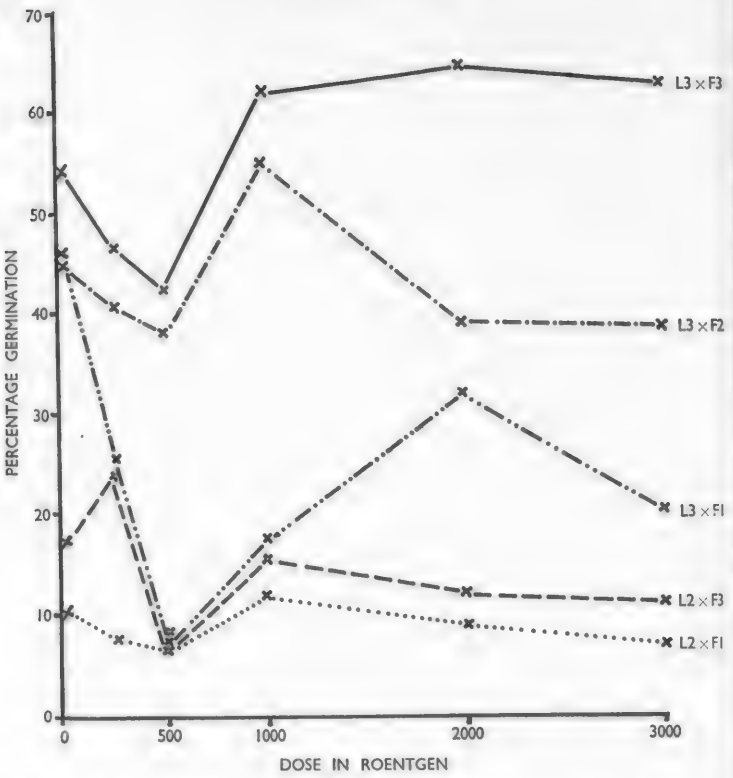

FIG. 1—The effect of pollen irradiation on seed-viability in crosses of *L. perenne* × *F. pratensis*.

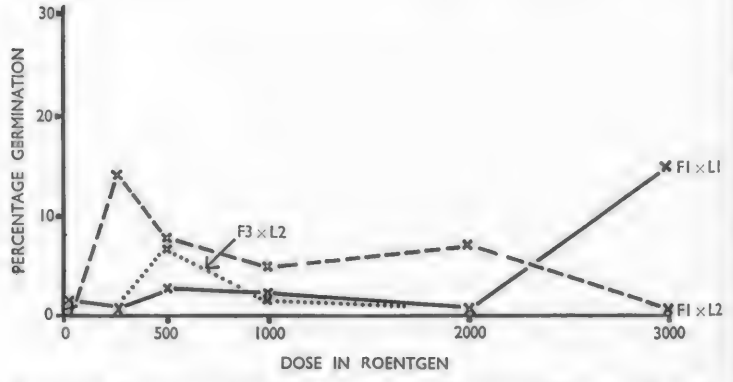

FIG. 2—The effect of pollen irradiation on seed-viability in crosses of *F. pratensis* × *L. perenne*.

of low compatibility (L2 × F1), irradiation had no significant effect; in the cross of medium compatibility (L2 × F3) and in one of the crosses of high compatibility (L3 × F1), a marked reduction in viability occurred at 500r, but the germination percentage at the higher doses did not exceed that observed in controls; in the remaining two crosses of high compatibility (L3 × F2 and L3 × F3) only a small reduction occurred at 500r. Moreover, in the latter combinations seed-viability at 1000r in the one case, and at doses higher than 1000r in the other, markedly exceeded that of the controls. Genotypic differences in the response to irradiation are therefore evident.

TABLE 2

The effect of ovule irradiation on seed-set and seed-viability in crosses between different genotypes of L. perenne *and* F. pratensis

Genotype combination	Percentage seed-set and germination at different doses of γ-rays						$\chi^2_{(5)}$
	Control	250r	500r	1000r	2000r	3000r	
L. perenne (♀) × *F. pratensis* (♂)							
L2 × F1 Seed-set	78·4	73·6	70·3	68·1	49·7	44·3	54·0 †
Germination	4·0	2·5	3·8	7·5	2·1	1·0	11·7 *
L3 × F3 Seed-set	78·5	66·4	65·5	51·0	37·8	34·2	141·3 †
Germination	59·4	48·0	51·7	27·1	18·1	16·0	97·0 †
F. pratensis (♀) × *L. perenne* (♂)							
F1 × L2 Seed-set	57·0	55·9	53·3	46·0	47·0	42·5	24·7 †
Germination	1·3	6·3	7·0	3·2	1·0	4·7	16·1 †
F3 × L3 Seed-set	62·5	46·5	42·7	40·5	33·9	27·3	77·2 †
Germination	0·0	0·0	0·0	0·0	0·0	0·0	...

* P = 0·05-0·01. † P = 0·01.

In the crosses with *F. pratensis* as seed parent (fig. 2), two factors partially conceal the effects of irradiation. Firstly, crosses showing a high degree of seed-compatibility where *L. perenne* is used as seed parent are not the most successful combinations in reciprocal matings. Secondly, the reduction in seed-compatibility at 250-500r, if it does occur, cannot be observed, since no viable seeds are produced when untreated pollen is used. Nevertheless, there is still some evidence for genotypic differences in the response to irradiation, in that the greater the genetic difference between genotypes, the greater was the dose required for optimum viability. For example, L1 and F1 are, on the basis of *L. perenne* × *F. pratensis* crosses, more incompatible, and hence probably more divergent genetically, than L2 and F1. In the former, the greatest viability was obtained at 3000r, in the latter at 250-500r.

(b) *The effect of ovule irradiation on compatibility*

Table 2 and fig. 3 show results on the effect of ovule irradiation for two series of reciprocal crosses. In both *L. perenne*×*F. pratensis* and *F. pratensis*×*L. perenne* crosses, seed-set decreased with dose. A similar overall response was observed for seed-viability in the *L. perenne*×*F. pratensis* combination L3×F3, the slight increase at 500r being non-significant. In L2×F1, on the other hand, viability at

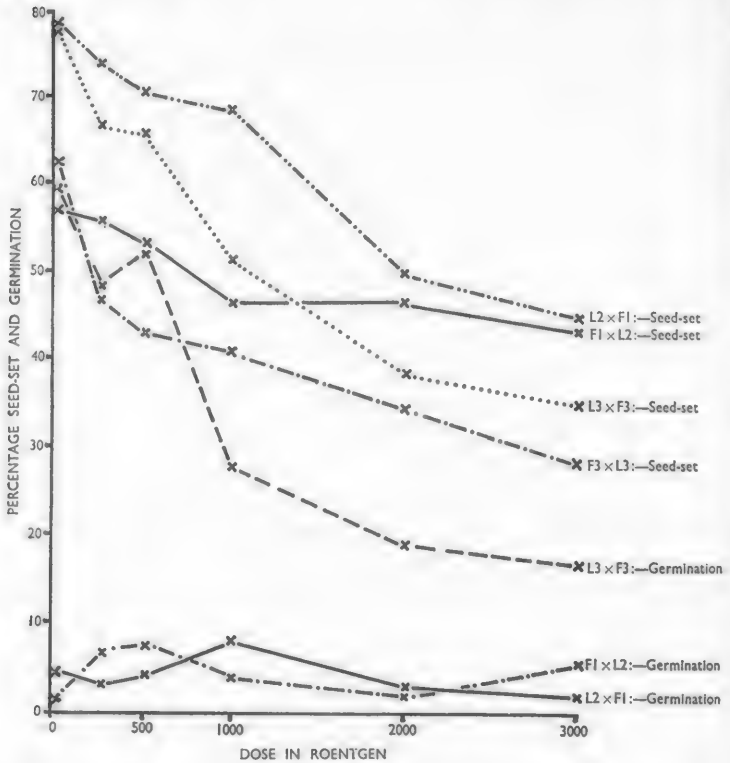

FIG. 3—The effect of ovule irradiation on seed-set and seed-viability in crosses between *L. perenne* and *F. pratensis*.

1000r was significantly higher than at 250r, but not significantly higher than in the control. It should be noted that normal viability in the latter cross was low compared with the former.

In one of the *F. pratensis*×*L. perenne* crosses analysed, namely F3×L3, ovule irradiation had no effect. In the other (F1×L2) a significant increase in viability occurred up to 500r, after which there was a decrease. The slight increase at 3000r was not significant.

(c) *Endosperm development following irradiation*

In order to determine the nature of the effect of irradiation on seed-compatibility, a single cross, namely L3 × F3, was examined embryologically following the use of pollen treated with 3000r. In this combination, as shown above, seed-viability at the higher doses of irradiation was greater than that where untreated pollen was used. Table 3 gives the mean number of endosperm nuclei at fixed intervals after pollination. Results following the use of untreated F3 pollen and untreated intraspecific pollen are also given.

Up to 48 hours after pollination the rate of endosperm development in the two crosses did not differ from that in the control. By 72 hours, however, a stage at which precocious cell formation takes place in the endosperm of hybrid seeds (Reusch, 1959*b*) the number of endosperm

TABLE 3

Mean number of endosperm nuclei in the L. perenne *genotype L3, and in the cross L3 × F3 following pollination with both treated and untreated pollen*

Control and crosses	Mean number of endosperm nuclei at different times after pollination					
	12 hours	24 hours	48 hours	72 hours	96 hours	120 hours
L3—intraspecific control .	2·0	7·5	99·0	640·0	1414·0	2545·0
L3 × F3—untreated pollen .	2·0	8·5	101·0	454·0	809·0	1527·0
L3 × F3—pollen treated with 3000r	2·0	8·3	105·0	453·0	890·0	1775·0

nuclei in the crosses was lower than that in the control. It is significant that at 96 hours and later, this retardation in endosperm growth rate was less marked when using irradiated pollen.

4. DISCUSSION

The purpose of this investigation was primarily to determine the effect of irradiation on seed-compatibility, as measured by viability, but the results have shown clearly that seed-set is also markedly influenced and in a broadly similar manner. On the data available it is difficult to find any single satisfactory explanation for the observed responses. However, the almost regular reduction in seed-viability at the lower doses, followed by an increase, in those crosses where viable caryopses are produced, seem to indicate two separate effects of irradiation. The first effect causes a deterioration of seed-compatibility, while the second brings about an improvement. What is the nature of these effects? Since in crosses between *L. perenne* and *F. pratensis*, seed-incompatibility results from an impaired development

of the endosperm (Reusch, 1959*b*), it is evident that they must be concerned with the development of the endosperm and the following are suggested: (*a*) Up to 500r chromosomally damaged pollen grains are not affected in their functional ability so that they are able to participate in fertilisation and triple fusion. However, as a result of chromosomal abnormalities, degeneration of the embryo and/or endosperm of a proportion of the caryopses sets in soon after fertilisation in a manner similar to that reported in *Lilium formosum* (Cave and Brown, 1954). In consequence, many of the seeds will not be viable. This degeneration presumably accounts for the reduction in viability in crosses of *L. perenne* by *F. pratensis* following pollen treatment with doses of 250-500r, and is therefore not genic. With doses higher than 500r, the functional ability of the pollen grains is presumably affected, so that selective elimination of the more severely damaged ones will occur. In consequence there will be a tendency towards a levelling-off of the deleterious effect. In the case of ovules, selective elimination cannot occur, so that their functional ability (measured in terms of the number of ovules which set seed) should decrease with an increase of dose. This did occur. (*b*) The recovery in seed-compatibility at doses higher than 500r is due to a second influence of irradiation, which allows those caryopses, in which chromosomal damage does not lead to a cessation of growth, to develop more endosperm than seeds set following the use of untreated pollen. Since this beneficial effect of irradiation markedly varies with different genotypic combinations, it would appear that gene changes are involved, so that superimposed upon the initial non-genic deleterious effect there is a beneficial genic effect, the nature of which could be explained as follows:

If one accepts that two genotypes, which under normal conditions of interspecific cross-pollination give rise to only a few viable seeds, are genetically more diverse than two which yield a large proportion of germinable caryopses, then, on the basis of a threshold of gene changes for improvement of endosperm development, one would expect that a larger number of gene changes and hence larger dosages of irradiation would be required to bring about an improvement in seed-compatibility in the former than in the latter. That this is the case is clear from the *L. perenne* × *F. pratensis* crosses (see earlier). On this basis it is also to be expected that in crosses where initial differences are too great, irradiation-induced gene changes will be ineffective in reducing the genetic disparity between the genomes in the endosperm. This would then be the case in those combinations where irradiation had no effect on seed-compatibility.

Since the crosses of *F. pratensis* × *L. perenne* all produced no viable seeds under normal conditions, it is evident that the deleterious effects of irradiation discussed above cannot be detected. The beneficial effect, on the other hand, was clearly observable, in that a number of the combinations yielded viable seeds following irradiation of both pollen and ovules.

Following on the above, one further point needs consideration, namely the manner in which endosperm development is influenced. From the *L. perenne*×*F. pratensis* cross analysed, it does appear that the degree of suppression of nuclear division, following precocious cell formation, is mainly affected. In consequence a larger amount of endosperm tissue is formed following the use of treated pollen, than in cases where untreated pollen is used. Similar observations have been made on low×high chromosome species crosses of *Avena* and *Triticum*, where better-filled caryopses were obtained with X-irradiated pollen than with untreated pollen (Nishiyama and Iizuka, 1952).

For crosses of *F. pratensis*×*L. perenne* one must assume that the intensity of abnormal divisions within the endosperm are reduced.

5. SUMMARY AND CONCLUSIONS

1. Results are presented on the effect of gamma irradiation on seed-set and seed-viability in reciprocal crosses between *L. perenne* and *F. pratensis*.

2. Following irradiation of the pollen, a reduction occurred in hybrid seed set at doses of 250-500r. This was followed by an increase at 1000-2000r, but further increases in dose generally again resulted in a decline. Irradiation of ovules led to a progressive decrease in seed set with increase of dose.

3. In crosses of *L. perenne*×*F. pratensis*, pollen irradiation, in general, did not raise the germination capacity of the caryopses above that for crosses in which untreated pollen was used. All combinations which gave rise to viable seeds under normal conditions, showed some decrease after irradiation at 500r, followed by an increase at the higher doses. Both the degree of reduction at 500r and the increase at higher doses varied with the genotypes involved in the cross.

4. Where *F. pratensis* was the seed parent, no viable seeds were obtained after pollination with untreated pollen. In three combinations out of six, however, viable seeds were produced as a result of pollen irradiation. Here, genotypic differences in the response to irradiation were also apparent.

5. It is suggested that the initial reduction in seed-viability is a consequence of endosperm degeneration in a proportion of ovules due to fertilisation by chromosomally damaged pollen grains. The increase at the higher doses is considered to be due to gene changes which reduce genetic unbalance within the endosperm, thus allowing for an improved development of the tissue.

6. It may be concluded that through the agency of ionising radiations, it is possible, in certain instances at least, to reduce the degree of seed-incompatibility. The improvement in viability may, however, be mainly limited to crosses made in one direction. Thus in *L. perenne*× *F. pratensis* crosses, the germination capacity of the caryopses was generally not improved, and in incompatible combinations irradiation

had no effect. In reciprocal matings, on the other hand, viable seeds were obtained from combinations which after normal pollinations gave no germinable caryopses.

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6. REFERENCES

CAVE, M. S., AND BROWN, S. W. 1954. The nature and detection of dominant lethals in *Lilium* II. Cytological abnormalities in ovules after pollen irradiation. *Amer. J. Bot.*, *41*, 469-483.

NISHIYAMA, I., AND HIZUKA, M. 1952. Successful hybridisation by means of X-rayed pollen in otherwise incompatible crosses. *Bull. Res. Inst. Food Sci., Kyoto Univ.*, No. 8.

PETO, F. H. 1933. The cytology of certain intergeneric hybrids between *Festuca* and *Lolium*. *J. Genetics*, *28*, 113-156.

REUSCH, J. D. H. 1959*a*. The nature of the genetic differentiation between *Lolium perenne* and *Festuca pratensis*. *S. Afric. J. Agric. Sci.*, *2*, 271-283.

REUSCH, J. D. H. 1959*b*. Embryological studies on seed-development in reciprocal crosses between *Lolium perenne* and *Festuca pratensis*. *S. Afric. J. Agric. Sci.* In press.

REUSCH, J. D. H. 1959*c*. Note on the nature of the effect of the environment on seed-viability in interspecific hybrids. *S. Afric. J. Agric. Sci.* In press.

VALENTINE, D. H. 1953. Evolutionary aspects of species differences in *Primula*. *Symp. Soc. Exp. Biol.*, *7*, 146-158.

A SUPPRESSOR OF LEUCINELESS IN *SALMONELLA TYPHIMURIUM*

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1. INTRODUCTION

In experiments which were designed to measure the rate of mutation of a leucine auxotroph of *Salmonella typhimurium* to prototrophy it was found that the apparent " reversions " were of three types. These were distinguishable by their growth rates in the absence of leucine. This paper presents an analysis of one of these " reversions " which has a slower growth rate in the absence of leucine than the wild type.

2. TERMINOLOGY, MATERIALS AND METHODS

The terminology and nomenclature used is that described by Demerec (1956). The symbol for each mutant is an abbreviation of the nutritional requirement involved : *try*, requires tryptophane ; *me*, requires methionine ; *leu*, requires leucine ; *ara*, unable to utilise arabinose. Similar mutants of independent origin are distinguished by numbers, assigned to the mutants in the order in which they are found. When the locus of the mutant allele is known, a capital letter designating that locus is included in the symbol for the mutant ; e.g. *tryB*-2. The symbol for a suppressor gene includes an abbreviation of the mutant character it suppresses together with an identifying number ; e.g. *su-leu*-1 is the first isolated suppressor of leucine requirement. Symbols for wild type alleles are omitted except where ambiguity may arise, when the symbol for the mutant allele with a superscript plus sign is used.

The triple auxotrophs *leu*-151, *meA*-22, *tryB*-2 and *leu*-152, *meA*-22, *tryB*-2 were obtained from the double auxotroph *meA*-22, *tryB*-2 by irradiation with ultra-violet light followed by penicillin screening. The double mutant *leu*-39, *ara*-9 was obtained from the Cold Spring Harbor collection. All the stocks used were *S. typhimurium* strain LT-2.

Temperate phage PLT-22 was used in all transduction experiments.

Cultures were grown in 1 per cent. Difco nutrient broth and assayed on 2·55 per cent. Difco nutrient agar. The minimal media (MM) and enriched minimal media (EMM) were those of Yura (1956) with the addition of 0·002 per cent. of methionine and of tryptophane. Leucine supplemented media (MM+L and EMM+L) contained in addition 0·002 per cent. *dl*-leucine. For EMB agar, 0·04 per cent. eosin, 0·006 per cent. methylene blue and 1·0 per cent. arabinose were added to 2·55 per cent. nutrient agar.

T2 buffer contained KH_2PO_4 0·15 per cent., NaCl 0·4 per cent., K_2SO_4 0·5 per cent., Na_2HPO_4 0·3 per cent., $MgSO_4$ 0·012 per cent., $CaCl_2$ 0·0011 per cent. and gelatin 0·001 per cent.

2.1. *Preparation of phage*

The donor strain was grown for 24 hr. on a nutrient agar slope and the bacteria washed off in 5 ml. of 1 per cent. nutrient broth. Equal volumes of this suspension and a phage suspension with a titre of approximately 10^8 particles/ml. were mixed and stood for 8 min. to allow for adsorption of the phage, after which 0·1 ml. samples

of the mixture were added to 5 tubes, each containing 2-3 ml. of sterile 0·7 per cent. molten agar at 37°. The contents of each tube were shaken and poured over the surface of a nutrient agar plate. After the plates had been incubated for 24 hr. the sloppy agar was scraped off, shaken up in 15 ml. of T2 buffer and stood for 2 hr. The bacterial debris and agar were removed by centrifuging, and the supernatant sterilised by shaking with 1-2 ml. of chloroform. The preparation was stored at 4° until required, when air was bubbled through until all the chloroform had evaporated.

The phage preparations were assayed by the agar layer technique (Adams, 1950). Using the above method yields of $1\text{-}4\times10^{11}$ particles/ml. are obtained when a sensitive donor is used. With a lysogenic donor the yield is $10^{7}\text{-}10^{8}$ particles/ml.

2.2. *Transduction*

Bacteria from overnight broth cultures of the recipient were washed twice and suspended in T2 buffer. These suspensions had a titre of $2\text{-}3\times10^{9}$ bacteria/ml. The high titre phage preparations were diluted in T2 buffer to a titre of $2\text{-}3\times10^{10}$ particles/ml. and 1 ml. of this dilution added to 1 ml. of the suspension of the recipient, giving a multiplicity of infection of 10. After standing for 9 min. to allow the phage to be adsorbed, 0·1 ml. samples of the transduction mixture were spread on EMM. When a high frequency of transduction was expected, a 10^{-1} dilution of the transduction mixture was also plated. The controls, to determine the frequency of spontaneous mutations, were (*a*) 1 ml. of recipient plus 1 ml. of T2 buffer and (*b*) 1 ml. of recipient plus 1 ml. of homologous phage. The total numbers of viable bacteria in the transduction mixtures and controls were determined by diluting appropriately and plating on nutrient agar.

With the low titre phage preparations the same method was used except that the suspension of the recipient was diluted 1 in 40 or 1 in 80, and the phage preparation was used undiluted.

All plates were scored after three days' incubation at 37°.

2.3. *Testing of clones*

A proportion of the clones which arose by transduction and by mutation were tested to determine their growth rates in the presence and absence of leucine. Clones were shaken in saline, diluted and plated on MM and MM+L. The plates were examined after 24 and 48 hr. incubation.

In transductions between arabinose fermenting (*ara*$^{+}$) and arabinose non-fermenting (*ara*-9) stocks, the clones were tested for their ability to ferment arabinose by streaking on EMB agar.

3. THE GENETIC DIVERSITY OF THE MUTANTS

When spontaneous mutations of *leu*-151 to leucine independence are selected by plating large numbers ($10^{8}\text{-}10^{9}$) of *leu*-151 bacteria on EMM, the resulting clones are of three types. For convenience these have been called fast, slow and unstable, and are distinguishable by their growth when subcultured on media with and without leucine (Smith-Keary, 1958). Both the fast and slow types are stable; the unstable type is characterised by a very high rate of reversion to auxotrophy and will not be considered further in this paper.

When synthetic liquid minimal media was inoculated with bacteria from a 24-hour slope, the fast and wild types each had a lag phase of 180 minutes followed by logarithmic growth with a doubling time of 46 minutes. The slow type had a lag phase of 240-300 minutes

and a doubling time during the logarithmic growth of 71·4 minutes. The cultures were grown at 37° with aeration.

Phage raised on the fast and slow growing types was used to infect the auxotroph *leu*-151 and transductions to prototrophy selected by plating on EMM. The results of these transductions for two different experiments are given in table 1. The fast and slow type donors yielded only fast and slow type transductional clones respectively; these are phenotypically similar to the fast and slow type clones arising by spontaneous mutation from *leu*-151. The fast and slow characters are transduced at different frequencies. In this and in subsequent experiments the frequency of transduction, expressed as the number of transductions per 10^8 survivors, was calculated from the formula

$$t = 100\left(\frac{n}{py} - \frac{m}{qz}\right)$$

TABLE 1

Transduction of leu-151 *to the fast and slow types*

Expt.	Donor	1 Bacteria plated $\times 10^{-7}$	2 Percentage of survivors	3 Transductions + spontaneous mutations observed	4 Spontaneous mutations/ 10^8 survivors	5 Transductions/ 10^8 survivors	6 Clones tested	7 Types of clones tested
A	fast	7	20·7	690	7·1	4751·9	30	all fast
	slow	7	22·8	229	7·1	1423·9	30	all slow
B	fast	4·76	16·8	428	5·8	5344·2	30	all fast
	slow	4·76	11·2	153	5·8	2848·2	30	all slow

where t is the number of transductions per 10^8 survivors, n and m are the observed numbers of clones on the transduction and control plates, p and q are the percentages of survival of the transduction mixture and the control, and y and z are the numbers of bacteria $\times 10^{-8}$ plated in the transduction and the control.

These results suggest that the fast and slow types are genetically different and that the slow type is not due to either a cytoplasmic factor or to a non-genetic difference.

4. THE GENETIC MODELS

Since the fast type, whether obtained by transduction or by spontaneous mutation, is indistinguishable from the wild type it is reasonable to interpret it as being due to a true backmutation of *leu*-151 to wild type. Three models are possible to explain the slow type reversions:— (1) there has been a mutation at the *leu*-151 site to an allele other than *leu*$^+$; (2) there has been a mutation at a second locus (*i.e.* a suppressor

mutation) which enables the requirement for leucine to be partially overcome; (3) the slow type carries the *leu*$^+$ allele but an inhibitor is present which partially inhibits the synthesis of leucine. These alternatives are set out below, together with the inferred genetic constitutions of the auxotroph and the fast and slow types;

	1.	2.	3*a*.		3*b*.
Fast or wild type	*leu*$^+$	*leu*$^+$ *su-leu*$^+$	*leu*$^+$ *I*$^+$		*leu*$^+$ *I*$^+$
Auxotroph	*leu*-151	*leu*-151 *su-leu*$^+$	*leu*-151 *I*$^+$	or	*leu*-151 *I*
Slow type	*leu-sl*	*leu*-151 *su-leu*	*leu*$^+$ *I*		*leu*$^+$ *I*

Models 3*a* and 3*b* are unlikely since a double mutation is required for *leu*-151 to mutate to the slow type (3*a*) or to the fast type (3*b*).

Provided that a fast growing type can be obtained from the slow type, either by mutation or by transduction, it should be possible to distinguish between models 1 and 2 by using the method devised by Yura (1956). If the suppressor hypothesis is correct, it should be possible to obtain a type with the genotype *leu*$^+$ *su-leu* from the slow type either by mutation of *leu*-151 to *leu*$^+$, or by transduction using wild type (*leu*$^+$ *su-leu*$^+$) phage, provided that *leu*$^+$ *su-leu* has a faster rate of growth on MM than has *leu*-151 *su-leu*. By raising phage on *leu*$^+$ *su-leu* and using it to infect the auxotroph *leu*-151 *su-leu*$^+$, both *leu*$^+$ *su-leu*$^+$ (fast) and *leu*-151 *su-leu* (slow) clones should be recovered as a result of transduction of *leu*$^+$ and *su-leu* respectively, provided that the suppressor and leucine loci are not very closely linked. Alternatively, if model 1 is correct, only fast type clones would be recovered.

5. THE GENETIC TESTS

Attempts to isolate a fast growing type from the slow type by mutation were unsuccessful. Several clones were isolated which had a growth rate intermediate between that of the slow and fast types, but transduction experiments showed these to be genetically similar to the slow type.

An attempt was then made to produce the fast type (*leu*$^+$ *su-leu* or *leu*$^+$) by transduction. Phage raised on a wild type donor was used to infect a slow type recipient and the transduction mixture spread on MM plates. After 24-hour incubation three of the largest transduced clones were picked off and subcultured; all had a growth rate similar to that of the fast type. These fast type clones will be referred to as fast′ 1, 2 and 3.

Phage was raised on each of these lysogenic fast′ types; the preparations had a titre of approximately 10^7 particles/ml. Care was taken to ensure that no potentially transducing phage was carried over from the phage inoculum by growing the phage on sensitive auxotroph bacteria immediately prior to growing it on the fast′ types. Each of the preparations was used to infect a dilute suspension of the auxotroph *leu*-151 and transductions to prototrophy selected by plating on EMM. The results of these experiments are given in

TABLE 2

Transductions using the fast′ type as donor and leu-151 as recipient

TABLE 3

Transductions using the fast' type as donor and leu-*151 as recipient*

Expt.	Donor	1 Bacteria plated $\times 10^{-7}$	2 Percentage of survivors	3 Transductions +spontaneous mutations observed	4 Spontaneous mutations observed	5 Transductions/10^8 survivors	6 Clones tested			7 Clones expected by spontaneous mutation			8 Clones arising by transduction	
							Total	Fast	Slow	Total	Fast	Slow	Fast	Slow
C	Fast' 1	5·6	100	153	...	266·1	70	63	7	1·82	0	1·82	63	5·18
	Control	14·0	100	...	10	...	40*	0	40	...	...	...	...	...
D	Fast' 2	1·26	43·9	42	...	675·7	36	23	13	3·95	1·48	2·47	21·52	10·53
	Fast' 3	1·26	31·7	33	..	742·7	33	20	13	3·33	1·25	2·08	18·75	10·92
	Control	0·72	100	...	6	...	40*	25	15	...	...	...	...	...

* Obtained from additional control.

TABLE 4

Transductions using su-leu-*1* leu$^+$ *as donor and* leu-*151*, leu-*152* and leu-*39* *as recipients*

Expt.	Recipient	1 Bacteria plated $\times 10^{-7}$	2 Percentage of survivors	3 Transductions +spontaneous mutations	4 Spontaneous mutations observed	5 Transductions/10^8 survivors	6 Clones tested			7 Clones expected by spontaneous mutation			8 Clones arising by transduction	
							Total	Fast	Slow	Total	Fast	Slow	Fast	Slow
E	*leu*-151	1·68	66·6	99	...	747·0	40	25	15	6·2	0	6·2	25	8·8
	leu-151 control	1·68	100	...	23	...	40*	0	40	...	...	...	...	...
F	*leu*-152	1·05	80·9	60	...	590·2	40	28	12	6·51	0·32	6·19	27·68	5·81
	leu-152 control	1·47	100	...	17	...	40*	2	38	...	...	...	...	...
G	*leu*-39	3·16	48·1	46	...	302·6	40	40	0	0	0	0	40	0
	leu-39 control	3·16	100	...	0	...	0	0	0	...	...	...	...	...

* Obtained from additional controls.

table 2. In each experiment there is an excess of both the fast and slow type clones over the number expected by spontaneous mutation (table 2, col. 7). These can only have arisen by transduction and so the suppressor hypothesis is confirmed. Hence the genetic constitution of the auxotroph is *leu*-151 *su-leu*-1$^+$, the slow type is *leu*-151 *su-leu*-1, the fast type is *leu*$^+$ *su-leu*-1$^+$ and the fast' type is *leu*$^+$ *su-leu*-1.

6. THE SPECIFICITY OF THE SUPPRESSOR *su-leu*-1

Three leucine auxotrophs were tested in combination with *su-leu*-1; *leu*-151 and *leu*-152 (independently isolated from *meA*-22 *tryB*-2) and *leu*-39. In reciprocal transductions between *leu*-151 and *leu*-152 the yield of prototrophic clones was of the same order as when the recipients were treated with homologous phage, and not in excess of the number expected by spontaneous mutation. Thus *leu*-151 and *leu*-152 are

TABLE 3

Number of prototroph clones per 10^8 survivors arising in transductions between leu-*151*, leu-*152*, leu-*39 and the wild type*

Donor / Recipient	*leu*-151	*leu*-152	*leu*-39	WT
leu-151	6·2	7·8	415	4758
leu-152	5·9	7·2	309	3488
leu-39	362	276	0	3816

either identical alleles or mutants at very closely linked sites. In reciprocal transductions between *leu*-151 or *leu*-152 and *leu*-39 the frequency of prototrophic clones was much higher than could be accounted for by spontaneous mutation, but lower than when phage raised on wild type bacteria was used (table 3). No cross-feeding occurred between *leu*-151 and *leu*-39. Hence *leu*-151 or *leu*-152 and *leu*-39 are probably mutants at linked but different sites of the same leucine locus.

When phage raised on fast' 2 (*leu*$^+$ *su-leu*-1) was used to infect *leu*-151 and *leu*-152 an excess of both fast and slow type clones over the number expected by spontaneous mutation was obtained. When *leu*-39 was similarly infected only fast clones were recovered (table 4). *su-leu*-1 is therefore allele specific. That both *leu*-151 and *leu*-152 are suppressed by *su-leu*-1 is further evidence that they are identical alleles.

7. THE ORDER OF THE GENETIC MARKERS

Since the leucine and arabinose loci are linked (Demerec *et al.*, 1958) the position of *leu*-151 relative to *leu*-39 and *ara*-9 could be determined by using *ara*-9 and *ara*$^+$ as unselected markers in reciprocal

transductions between *leu*-151 *ara*$^+$ and *leu*-39 *ara*-9, selecting for leucine independence. The resulting clones were streaked on EMB agar to determine whether they were *ara*$^+$ or *ara*-9. Table 5 shows the percentages of *ara*$^+$ and *ara*-9 clones among the leucine independent clones from the transductions *leu*-151 *ara*$^+$(×)*leu*-39 *ara*-9, *leu*-39 *ara*-9(×) *leu*-151 *ara*$^+$ and *leu*-39 *ara*-9(×)*leu*$^+$ *ara*$^+$.

Table 6 shows the regions of the recipient chromosome and the transduced fragment in which crossing over must occur in order to give *ara*$^+$ and *ara*-9 leucine independent clones in the transductions *leu*-39 *ara*-9(×)*leu*-151 *ara*$^+$ and *leu*-151 *ara*$^+$(×)*leu*-39 *ara*-9, for each of the three possible orders of the markers (1) *leu*-39 *leu*-151 *ara*-9, (2) *leu*-151 *leu*-39 *ara*-9, and (3) *leu*-151 *ara*-9 *leu*-39.

If the order were *leu*-39 *leu*-151 *ara*-9, the transduction *leu*-39 *ara*-9(×) *leu*-151 *ara*$^+$ would yield *ara*$^+$ clones only as a result of quadruple

TABLE 5

Results of transductions between leu-*151* ara$^+$, leu-*39* ara-*9 and wild type*

Expt.	Transduction	1 *leu*$^+$ clones per 10^8 survivors	2 Clones tested	3 Clones *ara*$^+$	4 Clones *ara*-9	5 Percentage of clones *ara*$^+$	6 Percentage of clones *ara*-9
H	*leu*-151 *ara*$^+$ (×) *leu*-39 *ara*-9	255	194	190	4	97·9	2·1
I	*leu*-39 *ara*-9 (×) *leu*-151 *ara*$^+$	256	160	99	61	61·9	38·1
J	*leu*-39 *ara*-9 (×) *leu*$^+$ *ara*$^+$	2949	160	109	51	68·1	31·9

crossing over, and a large deficiency of this class would be expected compared with the frequency of *ara*$^+$ clones which arise when wild type bacteria are used as the donor strain. Such a deficiency was not found.

If the order were *leu*-151 *leu*-39 *ara*-9, *ara*-9 clones from the transduction *leu*-151 *ara*$^+$(×)*leu*-39 *ara*-9 could only arise by quadruple crossing over, and a large excess of *ara*$^+$ clones would be expected. This is in agreement with the observation that 97·9 per cent. of the clones were *ara*$^+$. If the order were *leu*-151 *ara*-9 *leu*-39 this excess of *ara*$^+$ clones could only be accounted for if *ara*-9 and *leu*-39 were very closely linked and nearly always jointly transduced. That this is not so is evident from the transduction *leu*-39 *ara*-9(×)*leu*$^+$ *ara*$^+$, in which 31·9 per cent. of the leucine independent clones were *ara*-9. Furthermore, it is most improbable that the *ara* locus is between what are believed to be two alleles of *leu*.

When *leu*-39 *ara*-9 bacteria were transduced with phage raised on the slow type *leu*-151 *su-leu*-1 *ara*$^+$, both fast and slow type clones were recovered. 55·7 per cent. of these slow type clones were *ara*$^+$ (table 7). Since *su-leu*-1 is specific for *leu*-151 the *ara*$^+$ slow type clones can only

have arisen by the simultaneous transduction and incorporation of *leu*-151, *leu*-39$^+$, *su-leu*-1 and *ara*$^+$, and *su-leu*-1 must be linked to the *leu* and *ara* loci. The position of *su-leu*-1 relative to *leu* and *ara* loci can now be determined.

If the *ara* locus were between *leu*-151 and *su-leu*-1, all the slow type clones would be *ara*$^+$, except for a small number arising by quadruple crossing over. 44·3 per cent. of the slow type clones were *ara*-9, so that the order of the markers is not *leu*-151 *leu*-39 *ara*-9 *su-leu*-1. In 55·7 per cent. of the transductions of *su-leu*-1, *ara*$^+$ is also transduced; in 68·1 per cent. of the transductions of *leu*$^+$, *ara*$^+$ is also transduced. Hence it is probable that *su-leu*-1 is farther from the *ara* locus than is the *leu* locus, and the most likely order of the markers is *su-leu*-1 *leu*-151 *leu*-39 *ara*-9.

This order was confirmed by testing nine of the fast type clones from the transduction *leu*-39 *ara*-9(×)*leu*-151 *su-leu*-1 *ara*$^+$ to determine which of the alleles *su-leu*-1 and *su-leu*$^+$ they carried. Table 8 indicates the regions of the bacterial chromosomes in which crossovers must occur in order to give each of the four possible types of fast clones, *leu*$^+$ *su-leu*-1 *ara*-9, *leu*$^+$ *su-leu*-1 *ara*$^+$, *leu*$^+$ *su-leu*$^+$ *ara*-9 and *leu*$^+$ *su-leu*$^+$ *ara*$^+$. If the order of the markers is *su-leu*-1 *leu*-151 *leu*-39 *ara*-9, all the fast type clones should carry *su-leu*$^+$ except for those arising by rare quadruple crossing over. If the order is *leu*-151 *leu*-39 *su-leu*-1 *ara*-9 all the fast type *ara*$^+$ clones will carry *su-leu*-1, and the fast type *ara*-9 clones either *su-leu*-1 or *su-leu*$^+$, whilst if the order *leu*-151 *leu*-39 *ara*-9 *su-leu*-1 is correct, all the *ara*-9 fast type clones will carry *su-leu*$^+$ and the *ara*$^+$ fast type either *su-leu*-1 or *su-leu*$^+$.

Phage was raised on six fast type *ara*$^+$ and three fast type *ara*-9 clones and used to infect the auxotroph *leu*-151. In none of the transductions was there an excess of the slow type over the number expected by spontaneous mutation. Hence all of the nine fast type clones carry the wild type suppressor allele. This result can only be accounted for if the order of the markers is *su-leu*-1 *leu*-151 *leu*-39 *ara*-9.

8. DISCUSSION

The occurrence of suppressor mutations in *Salmonella typhimurium* was first demonstrated by Yura (1956) who showed that two sizes of mutant colonies appeared when the adenine-thiamine mutant *athA*-2 was plated on enriched minimal medium. The large colonies arose by back mutation at the *athA* locus while the small colonies arose by mutation at another, suppressor, locus. Mutations at this second locus (*su-ath*-1) specifically suppressed *athA*-2 and its identical allele *athA*-1. He also demonstrated the existence of a second suppressor mutation (*su-ad*-1) which was specific for the adenine mutant *adC*-11.

Similarly Howarth (1958) detected suppressors of cystine mutants. Five independent occurrences of suppressor mutations in mutants of the *cysA* and *cysF* loci, were each shown to suppress all known mutants

TABLE 6. *Showing the regions of the chromosome and transduced fragment in which crossing over must occur to give leucine independent* ara$^+$

TABLE 6.—*Showing the regions of the chromosome and transduced fragment in which crossing over must occur to give leucine independent* ara+ *and* ara-9 *clones in the transductions* leu-*39* ara-*9* (×) leu-*151* ara+ *and* leu-*151* ara+ (×) leu-*39* ara-*9, when the order of the markers is* (1) leu-*39* leu-*151* ara-*9*, (2) leu-*151* leu-*39* ara-*9 and* (3) leu-*151* ara-*9* leu-*39*

Transduction	*ara* genotype of *leu*+ clones	Percentage of *ara*+ and *ara*-9 clones	Cross over regions when the order of the markers is		
			leu-39 *leu*-151 *ara*-9 (1 2 3 4)	*leu*-151 *leu*-39 *ara*-9 (1 2 3 4)	*leu*-151 *ara*-9 *leu*-39 (1 2 3 4)
leu-39 *ara*-9 (×) *leu*-151 *ara*+	*ara*+	61·9	1-2 and 3-4	2-4	1-3
	ara-9	38·1	1-2	2-3	1-2
leu-151 *ara*+ (×) *leu*-39 *ara*-9	*ara*+	97·9	2-3	1-2	3-4
	ara-9	2·1	2-4	1-2 and 3-4	2-4

TABLE 7.—*Results of the transduction* leu-*39* ara-*9* (×) leu-*151* su-leu-*1* ara+

1 Transductions per 10^8 survivors	2 Leucine independent clones tested	3 Classification of clones tested				4 Percentage of *ara*+ and *ara*-9 clones in the fast and slow classes			
		Fast		Slow		Fast		Slow	
		ara+	*ara*-9	*ara*+	*ara*-9	*ara*+	*ara*-9	*ara*+	*ara*-9
104·9	231	7	7	121	96	50	50	55·7	44·3

TABLE 8.—*Showing the regions of the chromosome and transduced fragment in which crossing over must occur to give each of the four possible* leu+ *genotypes in the transduction* leu-*39* ara-*9* (×) leu-*151* su-leu-*1* ara+ *when the order of the markers is* (1) su-leu-*1* leu-*151* leu-*39* ara-*9*, (2) leu-*151* leu-*39* su-leu-*1* ara-*9 and* (3) leu-*151* leu-*39* ara-*9* su-leu-*1*

Genotypes	Crossover regions when the order of the markers is		
	su-leu-1 *leu*-151 *leu*-39 *ara*-9 (1 2 3 4 5)	*leu*-151 *leu*-39 *su-leu*-1 *ara*-9 (1 2 3 4 5)	*leu*-151 *leu*-39 *ara*-9 *su-leu*-1 (1 2 3 4 5)
su-leu-1 *ara*-9	1-2 and 3-4	2-4	2-3 and 4-5
su-leu-1 *ara*+	1-2 and 3-5	2-5	2-5
su-leu+ *ara*-9	3-4	2-3	2-3
su-leu+ *ara*+	3-5	2-3 and 4-5	2-4

of these loci. These suppressors, which may be independent isolations of the same suppressor mutation, were not simultaneously transduced with *cysA* and *cysF* markers, and there is no evidence that they are linked to this region of the chromosome.

The present work demonstrates the existence of a suppressor *su-leu*-1 which suppresses the leucine mutant *leu*-151 and its presumed identical allele *leu*-152, but which does not suppress *leu*-39. That this suppressor is linked to the *leu* and *ara* loci is indicated by the many simultaneous transductions of *su-leu*-1, *leu*-151 and *ara*$^+$ in the transduction *leu*-39 *ara*-9(×)*leu*-151 *su-leu*-1 *ara*$^+$. On the other hand, *su-leu*-1 and *leu* cannot be very closely linked, or, as Yura (1956) has pointed out, only *su-leu*$^+$*leu*$^+$ fast type clones would have been recovered from the transduction *su-leu*-1 *leu*-151(×)*su-leu*$^+$ *leu*$^+$. Of the three fast' colonies analysed, fast' 2 and fast' 3 are undoubtedly *su-leu*-1 *leu*$^+$, and fast' 1 probably is.

su-leu is believed to be the first instance of a suppressor in a micro-organism which has been shown to be linked to the gene that it suppresses.

Suppressors, differing widely in their degree of specificity, have also been described in *Neurospora crassa*. Yanofsky (1952) and Yanofsky and Bonner (1955*a*) have described four suppressors which act by partially restoring tryptophane synthetase activity in mutants lacking this enzyme. One suppressor, *S*-2 is allele specific for *td*^{2}, the other three being able to suppress more than one *td* allele. They have also demonstrated that four specific suppressors of *td*^{2} are due to suppressor mutations at, at least, three separate loci (Yanofsky and Bonner, 1955*b*). Giles and Partridge (1953) have described a suppressor of inositolless which is also allele specific. Unspecific suppressors have been described by Lein and Lein (1952), Giles (1951), Mitchell and Mitchell (1952) and Strauss and Pierog (1954). None of these suppressors has been shown to be linked to the locus it suppresses.

The mode of action of *su-leu*-1 has not been analysed biochemically, but since it is allele specific it is improbable that it acts by opening up an alternative synthetic pathway. Such a mechanism has been proposed by Howarth (1958) for the unspecific suppressors of cystine mutants, and has been shown to be responsible for the suppression of acetate requirement in *N. crassa* (Strauss and Pierog, 1954). *su-leu*-1 probably acts by one of the mechanisms proposed by Gots (1956) for the suppressor of adenine requirement in *S. typhimurium*—namely by either restoring the activity of an inactive enzyme, or by removing an enzyme inhibitor.

9. SUMMARY

1. A slow growing " reversion " of *leu*-151 in *Salmonella typhimurium* has been analysed by transduction and shown to be due to a suppressor mutation, *su-leu*-1.

2. *su-leu*-1 is specific for *leu*-151 and its probably identical allele *leu*-152, and has no effect in combination with *leu*-39.

3. *su-leu-*1 is linked to *leu* and to *ara*.

4. Using *ara*-9 as an unselected marker the order of the markers has been shown to be *su-leu-*1 $\begin{pmatrix} leu\text{-}151 \\ leu\text{-}152 \end{pmatrix}$ *leu*-39 *ara*-9.

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10. REFERENCES

ADAMS, M. H. 1950. Methods of study of bacterial viruses. In *Methods in Medical Research*, vol. 2, ed. J. H. Comroe, Jr. The Year Book Publishers Inc., Chicago, pp. 1-73.

DEMEREC, M. 1956. Terminology and nomenclature. In *Genetic Studies with Bacteria, Carnegie Inst. Wash. Pub., 612*, 1-4.

DEMEREC, M., LAHR, E. L., MIYAKE, T., GOLDMAN, I., BALBINDER, E., BANIČ, S., HASHIMOTO, K., GLANVILLE, E. V., AND GROSS, J. D. 1958. Bacterial genetics. *Carnegie Inst. Wash. Year Book, 57*, 390-406.

GILES, N. H. 1951. Studies on the mechanism of reversion in biochemical mutants of *Neurospora crassa*. *Cold Spring Harbor Symp. Quant. Biol., 16*, 283-313.

GILES, N. H., AND PARTRIDGE, C. W. H. 1953. The effect of a suppressor on allelic inositolless mutants in *Neurospora crassa*. *P.N.A.S., 39*, 479-488.

GOTS, J. S. 1956. The biochemical nature of a suppressor mutation in a purine requiring mutant of *Salmonella typhimurium*. In *Genetic Studies with Bacteria, Carnegie Inst. Wash. Pub., 612*, 87-96.

HOWARTH, S. 1958. Suppressor mutations in some cystine-requiring mutants of *Salmonella typhimurium*. *Genetics, 43*, 404-418.

LEIN, J., AND LEIN, P. S. 1952. Studies on a suppressor of non-allelic acetate-requiring mutants in *Neurospora*. *P.N.A.S., 38*, 44-48.

MITCHELL, M. B., AND MITCHELL, H. K. 1952. Observations on the behaviour of suppressors in *Neurospora*. *P.N.A.S., 38*, 205-214.

SMITH-KEARY, P. F. 1958. An unstable mutation in *Salmonella typhimurium*. *Nature, 181*, 1672.

STRAUSS, B. S., AND PIEROG, S. 1954. Gene interactions ; the mode of action of the suppressor of acetate-requiring mutants of *Neurospora crassa*. *J. Gen. Microbiol., 10*, 221-235.

YURA, T. 1956. Suppressor mutations in purine requiring mutants of *Salmonella typhimurium*. In *Genetic Studies with Bacteria, Carnegie Inst. Wash. Pub., 612*, 63-75.

YANOFSKY, C. 1952. The effects of gene change on tryptophane desmolase formation. *P.N.A.S., 38*, 215-226.

YANOFSKY, C., AND BONNER, D. M. 1955*a*. Gene interaction in tryptophane synthetase formation. *Genetics, 40*, 761-769.

YANOFSKY, C., AND BONNER, D. M. 1955*b*. Non-allelic suppressor genes affecting a single *td* allele. *Genetics, 40*, 602-603 (abstract).

THE EVOLUTION OF DOMINANCE UNDER DISRUPTIVE SELECTION

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1. INTRODUCTION

In a paper on the effects of disruptive selection, Mather (1955) pointed out that if there are two optimum values for a character and all others are less advantageous or disadvantageous there will be disruptive selection which can lead to the evolution of a polymorphism. Sheppard (1958) argued that where such selection is effective and the change from one optimum value to the other is switched by a single pair of allelomorphs there will be three genotypes but only two advantageous phenotypes. Consequently if dominance were absent initially it would be evolved as a result of the disruptive selection, the heterozygote and one of the homozygotes both coming to resemble one of the two optimum phenotypes (see Ford, 1955, on *Triphæna comes*). Thoday (1959) has shown by means of an artificial selection experiment that, even when a character is, at the beginning, controlled polygenically (sternopleural chaeta-number in Drosophila) and there is 50 per cent. gene exchange between the "high" and "low" selected sub-populations, a polymorphism can evolve.

The most fully understood examples of disruptive selection (other than sex) are provided by instances of Batesian Mimicry, where there are a number of distinct warningly coloured species, acting as models, which are mimicked by the polymorphic forms of a single more edible species. Fisher and Ford (see Ford, 1953) have argued that a sufficiently good resemblance between mimic and model is not likely to arise by chance as the result of mutation. They therefore suggest that a mutant arises which gives a fair resemblance and that there is subsequent selection for modifiers which improve the resemblance controlled by the new mutant. However, if as Fisher (1928) believes, the effects of a new mutant are neither fully dominant nor fully recessive from the beginning, the modification of the phenotype towards more perfect mimicry would also involve the evolution of dominance since normally there will be only two optimum patterns, the mimetic and the original one.

2. MATERIAL

In the course of an investigation into the genetics of the highly polymorphic mimetic butterfly *Papilio dardanus* Brown, we have obtained information which gives data on the evolution of dominance in a polymorphic species. *P. dardanus* is found in the Ethiopian region and occurs in several distinct races whose names

and geographical distribution are given in fig. 1 (for a detailed review see the excellent paper of Ford, 1936). The races which particularly concern us in this paper are (1) the South African race *cenea* (not to be confused with the mimetic form of the same name, which is found commonly in South Africa and mimics *Amauris albimaculata* and *Amauris echeria*), (2) the West African race *dardanus*, (3) the transitional race *meseres*, and (4) the Kenya race *polytrophus*. All these will be collectively known as the main African stock in this paper, since there is some gene exchange between them. Besides these races we shall also be considering two isolated ones, *meriones* from Madagascar and *antinorii* from Abyssinia.

FIG. 1.

In *P. dardanus* the males are all rather similar in appearance having a black and yellow pattern which is non-mimetic and possessing " tails " on their hind wings, a character typical of the genus *Papilio*. In the main African stock the females are usually mimetic, always tailless, and, with the exception of some individuals in the *polytrophus* race, never have the yellow fluorescent pigment characteristic of the males. In Madagascar, as well as in race *humbloti* from Grand Comoro, the females are similar to the males in pattern, in possessing yellow fluorescent pigment and in being tailed. In Abyssinia all the females are tailed and the majority are like the males, but a minority have mimetic patterns.

3. METHODS

The main variation of wing pattern in the forms to be considered occurs in three areas on the forewing and two on the hindwing. For convenience of description these areas have been given numbers in fig. 2. Two other areas of interest are the cells of fore and hind wings which are also marked in the figure.

Besides the wing pattern, there is also variation in tail length and in order to measure this it is necessary to determine fixed points of reference on the hindwing. The three points, given in fig. 2 are, A at the apex of the cell nearest its point of attachment to the body, C the point where vein 4 reaches the margin of the wing and B the point where a straight line joining the distal end of veins 3 and 5 cuts vein 4. The length of the tail is taken to be the distance BC and the length of the wing to be BA. Because the edge of the wing is scalloped even insects with no tail have a small protuberance which is measured by BC.

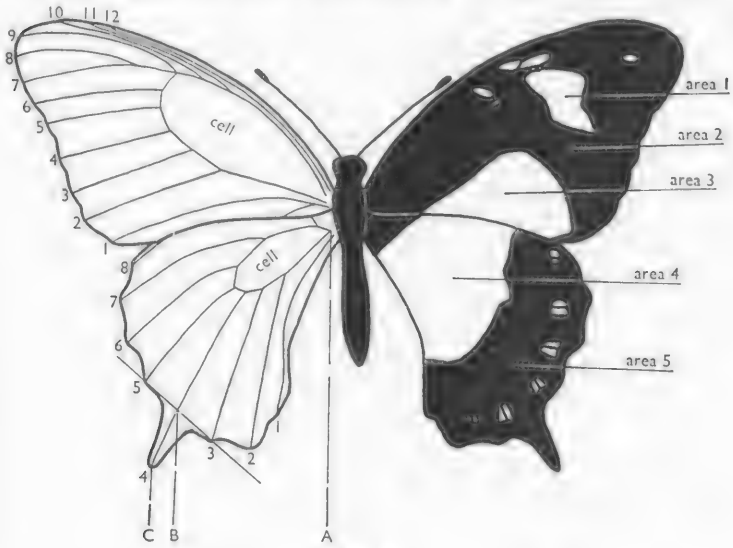

FIG. 2

Since the size of the hindwing varies from insect to insect, from sex to sex and from race to race and since there is a marked correlation between tail length, BC, and wing size, BA, it is necessary to adjust tail length to take into account differences in the size of the insects before comparisons can be made between them. This was done by calculating for each insect the factor which would adjust the length of BA so that it equalled the average (35·80 mm.) for all insects examined and correcting tail length BC by this same factor. For example if the length of BA for a particular insect were 38·50 mm. and the tail length were 9 mm. this value would be adjusted by the factor $\frac{35{\cdot}80}{38{\cdot}50}$ to give a length of 8·37 mm.

The procedure would not be legitimate if there were allometric growth. However, such a situation would lead to a correlation between adjusted tail length and unadjusted wing length and as no such correlation is apparent we can conclude that any allometric growth that there may be is too small to be detected in this material and that the procedure of adjusting tail length in accordance with the length of

the wing is a valid one. Not only does this adjustment reduce differences in mean tail length between the sexes and races but it also materially reduces the sample variance.

4. THE FEMALE FORMS

The female forms investigated by us, which occur together in each race (*i.e.* are sympatric) are given in table 1 together with some indication as to their frequency

TABLE 1

Race	Female form	Approximate frequency in per cent.	Model	Reference for frequency
cenea	*cenea*	40-85	*Amauris albimaculata*, *A. echeria*	F., C.
	hippocoonides	9-50	*A. niavius*	F., C.
	trophonius	4-15	*Danaus chrysippus*	F., C.
	natalica	0-10	None	F., C.
	leighi	<2	None	F., C.
	Imperfect-*trophonius*	1-0	? *D. chrysippus*	cal.
	salaami	<0·2	None	cal.
Transitional and *dardanus*	*hippocoonides* (*hippocoon*)	60-100	*A. niavius*	F.
	planemoides	0-40	*Bematistes poggei*	F.
	niobe	0-7	*B. tellus*	F.
	trophonius (*trophonissa*)	0-5	*D. chrysippus*	F.
	cenea	0-7	*A. echeria*, *A. albimaculata*	F.
	red-brown *cenea*	0-0·4	None	cal.
polytrophus	*cenea*	20-40	*A. echeria*, *A. albimaculata*	F.
	proto-*cenea*	5-15	? *A. echeria*, *A. albimaculata*	F.
	hippocoonides	20-40	*A. niavius*	F.
	yellow (proto-*hippocoonides*)	3-10	?*A. niavius*	F.
	pale-*poultoni* / bright-*poultoni*	5-15	None	F.
	trophonius	<1	*D. chrysippus*	F.
	proto-*trophonius*	5-15	? *D. chrysippus*	F.
	planemoides / proto-*planemoides*	5-15	*B. poggei* / ? *B. poggei*	F.
antinorii	yellow (male-like)	80-100	None	M.
	hippocoonides (*niavioides*)	0-20	*A. niavius*	M.
	trophonius (? *ruspinae*)	0-5	*D. chrysippus*	M.
	wienholti	0-1	? *B. epaea* and ? *Acraea jodutta*	M.
	cenea	0-1	*A. echeria*	M.
meriones	yellow (male-like)	100	None	F.

In the references C. stands for Clarke *et al.*, cal. for calculated, F. for Ford and M. for Museum and Mansfield collections.

and mimetic status. Where two colour patterns are controlled by the same main allelomorph but differ in appearance because of differences in the gene-complexes of the various races they have usually been given different names by entomologists.

In the table we have used one name for these genetically similar forms but have put in brackets the varietal name used by entomologists.

The patterns are described with reference to the areas marked on fig. 2. Owing to the difficulty of describing colours accurately, where necessary, the reference numbers of the colour of a typical specimen have been taken from Villalobos and Villalobos (1947) and are put in parentheses. The numbers are given in the order hue, value and degree respectively.

cenea (plate 3, no. 6) has the pale area 4 of the hindwing yellowish-buff (8-18-8) and the spots on the forewing are white to pale yellowish-buff.

hippocoonides (plate 3, no. 4) has all the pale areas white. The width of area 5 varies from race to race in parallel with that of a similar area on the local sub-species of the model.

trophonius has the same black pattern as *hippocoonides* except that area 5 is always narrow, as is a similar area on its model. Areas 3 and 4 are reddish-brown (7-16-12) and area 1 is white.

natalica has hindwings like those of *cenea*. The black pattern of the forewings is the same as that of *hippocoonides* but areas 1 and 3 are yellowish-buff to ochreous (8-19-9) like the hindwings and not white. Area 3 is often slightly reduced in size.

salaami is like *trophonius* except that area 1 is orange (7-17-10) not white.

pale-*poultoni* is like *salaami* except that all the pale areas are a very pale orange (7-17-9).

bright-*poultoni* is like *salaami* except that all the pale areas are a bright reddish-brown (6-15-12) being of a deeper hue than area 1 in *salaami* or *leighi*.

niobe has the same general pattern as *trophonius* except that area 1 is the same reddish-brown colour (6-15-12) as the rest of the pale areas. The body also has a decidedly reddish tint.

planemoides has hindwings like those of the local *hippocoonides*, the black in area 5 extending nearly to the cell. The black area 2 of the forewing is replaced by light orange-brown found also in 1 and 3 (7-15-11). Area 3 is reduced in size by the extension of the black pigment from the base of the wing. This black covers a triangular area extending from the base of the wing to about two-thirds of the way along the cell and halfway along the inner margin. Consequently though much of the wing is black there is a light orange-brown bar extending through areas 1, 2 and the distal end of 3.

leighi has a hindwing similar to that of *cenea* except that area 4 is often rather paler but never quite white. Area 1 is orange (7-17-10) and area 3 is black except for a large orange spot bordering area 2. A buff or white spot of the same size and shape and in the same position is found in *cenea* (plate 3, no. 6).

The male-like non-mimetic pattern of the female (plate 1, no. 1 ; plate 2, no 1) has the pale areas of the wings bright yellow (11 or 12-19-9) and the insect has tails. A fuller description of the forms can be obtained from Ford (1936) and the standard works listed by him.

5. RESULTS AND DISCUSSION

(i) *Dominance between sympatric forms*

The genetic work (Clarke and Sheppard, 1959*a*, 1959*b*, and in preparation) has shown that the forms *hippocoonides* (including *hippocoon* and *niavioides*), *natalica*, *cenea*, *trophonius*, *leighi*, *salaami*, pale-*poultoni*, and the male-like forms, henceforth called yellow, are all determined by an autosomal sex-controlled multiple allelomorphic series (or very closely linked genes) and it is also very probable, although not yet formally proved, that *planemoides*, *niobe* and bright-*poultoni* are governed

by allelomorphs at the same locus. Table 2 gives the female forms together with the allelomorphs and the genotypes that are known to give rise to them within their own race. It has been assumed for the purposes of this discussion that our interpretation of the genetic control of *niobe*, *planemoides* and bright-*poultoni* is correct. If this should prove not to be the case then the dominance relationships indicated by the tables should be reinterpreted as epistatic effects wherever these three forms are concerned. Reference to tables 1 and 2 will show that there are a number of allelomorph combinations which exist within the races but which we have not yet synthesised. The tables also show that a number of combinations will not occur in nature since the allelomorphs concerned are not found in the same race.

TABLE 2

Phenotypes	Allelomorph	Genotypes
hippocoonides . .	h	hh
cenea . . .	H^c	H^cH^c H^ch H^cH^{na}
natalica . . .	H^{na}	$H^{na}H^{na}$ $H^{na}h$
trophonius . .	H^T	H^TH^T H^Th H^TH^c
leighi . . .	H^L	H^LH^L H^LH^{na} H^LH^c H^Lh
salaami . . .	...	H^LH^T
imperfect *trophonius* .	...	H^TN^{na}
yellow . . .	H^y	H^yH^y H^yh H^yH^c
bright-*poultoni* . .	H^{bp}	$H^{bp}H^{bp}$ $H^{bp}h$ $H^{bp}H^T$
pale-*poultoni* . .	H^{pp}	$H^{pp}H^{pp}$ $H^{pp}h$
orange-*cenea* . .	...	$H^{bp}H^c$
buff-*cenea* . .	...	$H^{pp}H^c$
niobe . . .	H^{Ni}	$H^{Ni}H^{Ni}$ $H^{Ni}h$ H^TH^{Pl} $H^{Ni}H^c$ (some insects only)
red-brown *cenea* .	...	$H^{Ni}H^c$ (some insects only)
planemoides . .	H^{Pl}	$H^{Pl}H^{Pl}$ $H^{Pl}h$

It will be seen from table 2 that of the seven heterozygotes between allelomorphs both of which when homozygous control mimetic patterns only two give an intermediate heterozygote. These two exceptions are of interest in themselves. One, that between *planemoides* and *trophonius*, which will not be particularly rare (about 0·6 per cent. of the population near Entebbe), gives on most occasions an insect indistinguishable from *niobe* and therefore a mimic of *Bematistes poggei* and not at a disadvantage phenotypically. However, the heterozygote is rather more variable both in the exact colour of the red-brown areas and in the degree of development of the black bar (area 2) than in *niobe* itself suggesting that, at the lower frequency of the heterozygote, selection has not stabilised the pattern as it has in the much commoner *niobe* proper. The second exception is the heterozygote between *niobe* and *cenea* in which the insect sometimes resembles *niobe* and sometimes is distinguishable from it. In this instance there is no appropriate model and the heterozygote itself, when detectable, resembles a *cenea* with the pale areas red-brown instead of the normal

white and yellowish-buff. It will be moderately rare (about 0·4 per cent. or less) since neither of the allelomorphs in this heterozygote are common in races where both occur. Thus with the mimetic allelomorphs we find only one partial exception to the conclusion that disruptive selection will lead to the elimination of intermediates between optimum patterns.

When we examine the heterozygotes between allelomorphs, one of which controls a mimetic form and the other a non-mimetic one, we find there are thirteen in table 2 of which five are intermediate. These combinations in which dominance is absent are as instructive as the examples where both allelomorphs control mimetic patterns. Two of the five are not uncommon, one being the heterozygote between *cenea* and pale-*poultoni* and the other between *cenea* and bright-*poultoni* both of which resemble rather closely the *cenea/niobe* heterozygote. Now, except as great rarities, the allelomorphs H^{bp} and H^{pp} are only found in race *polytrophus* from Kenya, where the models for *P. dardanus* are uncommon and where consequently, the disruptive selection is much relaxed and many imperfect mimetic forms are found (Ford, 1936). The third is that between *trophonius* and pale-*poultoni* and gives a form which is almost identical to bright-*poultoni* and would therefore not be expected to be disadvantageous from the point of view of its appearance. The fourth example of an intermediate heterozygote is found in South Africa and is between *trophonius* and *natalica*. This heterozygote, although not particularly uncommon, comprising up to perhaps 1 per cent. of the female population in some areas, is also not a bad mimic of *Danaus chrysippus*. In fact it only differs from *trophonius* in having a pale buff instead of a white area near the apex of the forewing (fig. 2, area 1). In old specimens it is indistinguishable from *trophonius* and even in fresh specimens it is quite easy to score the insect as *trophonius*. In fact we have done this on one occasion and only discovered our mistake from the offspring of the mating. The last intermediate, *salaami*, also comes mainly from South Africa. Unlike the other forms it is very uncommon and cannot constitute more than 0·2 per cent. of the female population and may only account for 0·04 per cent. or less of the females in most populations. The form is very like bright-*poultoni* in appearance and quite unlike either homozygote (in addition to these five exceptions on rare occasions we have detected a modifier which makes *leighi* incompletely dominant to *cenea*). The breeding results, therefore, are consistent with the hypothesis that in mimicry, disruptive selection eliminates or materially reduces intermediate phenotypes except in the case of extremely rare heterozygotes or where the selection is weak or non-existent as in the *polytrophus* race.

There are two main hypotheses which could account for the absence of intermediate heterozygotes; (i) it could be argued that there was complete dominance from the first appearance of the mutant and that all disruptive selection can do is to stop allelomorphs with

intermediate phenotypes in the heterozygote from spreading; or (ii) that the dominance was not present initially but that it evolved as the result of selection for a modifier or modifiers. In most material it is impossible to gather data which will distinguish between these two alternatives but in *P. dardanus* it is possible to obtain information on the matter by making suitable race crosses. If the first hypothesis is correct, one would expect complete dominance in most instances when one introduced an allelomorph from one race into the gene-complex of another which does not and has not possessed it. On the second hypothesis one would expect a breakdown in dominance if this procedure were carried out.

(ii) *Absence of dominance between allopatric forms*

Now in the Madagascan race of *P. dardanus* all the females are male-like, having the presumed primitive pattern of the species. The F_1 hybrids between this race and the main African stock females (in this case race *cenea*) of the forms *hippocoonides*, *natalica* and *cenea* are intermediate in appearance having the pale areas on the wings yellow and a black pattern rather similar to that of *hippocoonides* (plate 1). Although the three genotypes give phenotypes which are very alike they are distinguishable from one another, each showing the effects of the particular allelomorph concerned. Thus the yellow areas in the *hippocoonides* heterozygote are a paler yellow (12-19-6) than either the Madagascan yellow (11 or 12-19-9) or that of the other two heterozygotes and in the first backcross to the main African stock they are even a little paler still (12-19-5 or 6). The *cenea* heterozygote, although it has much the same black pattern as in the *hippocoonides* hybrid, is recognisably different since the pale areas near the apex (area 1) and near the inner margin (area 3) of the forewing are smaller and the yellow is of a deeper hue (10-19-9). The *natalica* hybrid is also distinct, for although it has the same black pattern as the *hippocoonides* F_1 the yellow areas are of a deeper orange-yellow (8-18-8).

The only other heterozygote which we have produced, using the Madagascan race, concerns *trophonius*. The resulting phenotype is exceptionally variable. One of the extreme forms has the black pattern of *hippocoonides* but all pale areas orange-brown (areas 3 and 4, 7-16-11, area 1, 7-17-9), producing an insect very like *salaami*. The other is an almost exact replica of proto-*trophonius* from Kenya, having a tendency for the black bar (area 2) between the two pale areas (1 and 3) on the forewing to be reduced, the apical pale area pale yellow (12-19-3) not white, and a reduction of the area of red-brown in the forewing so that there is also an area of pale yellow near the reduced black bar (plate 2).

Since all four hybrid heterozygotes are dissimilar, and do not resemble the African homozygotes, or the Madagascan one, we can conclude with confidence that in the unadjusted gene-complex of the

hybrid dominance is absent. It is therefore very probable though not yet formally proved, that there is no dominance with respect to the four African allelomorphs and yellow in the pure Madagascan gene-complex. The only other possibility is that the Madagascan race possesses modifiers which confer dominance and that its absence in the F_1 hybrid is due to the recessiveness of some of these. The *trophonius* heterozygote is extremely variable. Since it is not variable in South African material the modifiers controlling the distribution of red pigment are likely to have come from the Madagascan race (see also the *antinorii* hybrids described below). This suggests that it is not well adjusted to this allelomorph. The *trophonius* homozygote in a Madagascan gene-complex can possess no yellow, since the allelomorph controlling this pigment will be absent, whereas the yellow homozygote can have no red. If the distribution of red is variable in the heterozygote, due to variations in the gene-complex, at least a proportion of the heterozygotes will be distinct from both homozygotes. Thus complete dominance will be absent.

It is also more than likely that the yellow pattern is not dominant in the main African stock since in the first backcross the *hippocoonides* yellow heterozygote is still much like the F_1 even though the *hippocoonides* characters are slightly enhanced.

Besides being the only one in Madagascar, the yellow pattern is also the commonest in Abyssinia. However, in Abyssinia, forms resembling *hippocoonides*, *trophonius* and *cenea* are also found (table 1). There is no report in the literature of *cenea* occurring in this area, but Mr Carcasson of the Coryndon Museum tells us that he saw a tailed form of *cenea* flying in Southern Abyssinia and Mr P. R. A. Mansfield of the British Embassy at Addis Ababa has sent us a photograph of a *cenea* with short tails which was caught near Filfil in Eritrea.

Similar crosses to those with the Madagascan race have been made using Abyssinian material but the results are quite different, as might be anticipated on our hypothesis, since some of the allelomorphs are common to both the Abyssinian and main African races. The only two Abyssinian phenotypes we have so far used are the yellow and *hippocoonides* forms. We found that the *natalica*/*hippocoonides*, *cenea*/*hippocoonides* and *trophonius*/*hippocoonides* heterozygotes produced normal *natalica*, *cenea* and *trophonius* respectively. However, the interesting heterozygotes from the point of view of comparison with the Madagascan crosses are the ones with the yellow allelomorph. The *hippocoonides*/yellow heterozygote is indistinguishable from the pure Abyssinian yellow form except that the black areas on the hindwing are joined up into a black border (area 5) as in *hippocoonides*. The *cenea*/yellow heterozygote is identical with the previous heterozygote except that the yellow is of a deeper hue (8-17-9) and a few black scales are sometimes found in area 2. The deeper yellow can also occur in some *hippocoonides*/yellow heterozygotes (plate 2). Thus, in the F_1 the dominance of the yellow pattern over *hippocoonides* and *cenea* is almost complete

and, after one backcross to the Abyssinian race, dominance is complete in both forms since even the black pattern of the hindwing is identical with that of the Abyssinian yellow form and the deeper yellow shade is also absent (plate 3). On backcrossing the yellow *hippocoonides* heterozygote into a *hippocoonides* strain of the main African stock, the *hippocoonides* characters become more and more enhanced and in the second and third generations the yellow insects are almost identical with those of the Madagascan race hybrids of the first backcross (plate 2).

The *natalica*/yellow F_1 heterozygote (8-17-8) is almost identical with the *cenea*/yellow heterozygote except that there is a strong tendency for the black bar, area 2, near the apex of the forewing to appear (plate 2). Thus the dominance of the yellow form is less complete in this heterozygote.

The results so far are in accord with the hypothesis that disruptive selection has built up the dominance of yellow in the polymorphic Abyssinian race but not in the monomorphic Madagascan one. Even the less complete dominance of yellow over *natalica* is in accord with this view since *natalica* is not known in the Abyssinian race. The results with *trophonius* also help to confirm the hypothesis although at first sight they might be thought to contradict it. Despite the fact that a *trophonius*-like form is found in Abyssinia (table 1) our *trophonius*-yellow heterozygote is intermediate between *trophonius* and yellow. The insects are variable but they have the hindwing pattern of *trophonius*. In the forewing the red-brown pigment is present in its normal position but it is also found to a variable degree in the normally white area near the apex of the wing (area 1), the rest of this area being yellow. The normal black bar (area 2) between these two pale areas is absent and replaced by yellow (plate 2). Thus the heterozygote is similar to the same heterozygote in the Madagascan race-cross, except for the absence of the black bar, and dominance is absent.

In all collections from Abyssinia no insect remotely resembling this form has ever been found despite the fact that several *trophonius*-like insects have been caught. Now the heterozygote will be much commoner than the rarer homozygote so that we must either conclude (i) that the *trophonius* form in Abyssinia is not controlled by the same gene as is the South and Central African form, in which case the intermediate heterozygote is just what would be expected on the hypothesis we are examining, or (ii) that on backcrossing into the Abyssinian gene-complex the form would either become completely dominant or completely recessive, which would also fit the hypothesis.

Three other allelomorphs, not known to occur in race *antinorii* and not included in the Madagascan race-crosses, have been used in making Abyssinian hybrids. The first H^L produces an insect resembling *leighi* in the $H^L h$ F_1 hybrid but in later generations (first backcross to main African stock and F_2) the heterozygotes were found to produce rather variable results, some insects being like *planemoides* and others

like *leighi*, the rest having intermediate patterns. No. F_1 hybrid heterozygote between *leighi* and *cenea* ($H^L H^c$) has been made. However, this heterozygote has been produced in the first and second backcross to the main African stock and in both broods it is quite distinct from either *leighi* or *cenea*. It has the *leighi* colouring and pattern save that the pale area near the apex of the forewing (area 1) is reduced in size and shows a tendency to be divided by black into several distinct spots. Thus on introducing part of the gene-complex of *antinorii* into the main African stock the complete dominance of *leighi* over *cenea* is removed. The *leighi*/yellow heterozygote is variable and intermediate in appearance between *leighi* and yellow. It resembles the *hippocoonides*/yellow F_1 hybrid except that area 1 has a marked orange-red flush due to the presence of many orange scales, there is sometimes a reduced black bar in area 2 and there are some orange scales in area 3 bordering both area 2 and the cell.

The second allelomorph is H^{bp} which controls the bright-*poultoni* pattern. The phenotype of the heterozygote with yellow, $H^{bp}H^y$ is also intermediate between the two homozygotes since the distribution of black pigment is the same as that in the other hybrids (area 2 being pale not black) but the yellow areas are replaced by red-brown (7-15-12). This insect is an almost exact replica of the form *dorippoides* which is only found, as a rarity, in East Africa. The $H^{bp}h$ heterozygote also looks like *dorippoides* (though less extreme) so that we cannot say whether the dominance has broken down or not, only that in the heterozygote the normal bright-*poultoni* phenotype has been altered. In pale-*poultoni* we know that the heterozygote with yellow is intermediate in appearance. Thus all our results so far reported are consistent with the presence of dominance between forms which are sympatric, common and subject to strong disruptive selection and its absence between most allopatric forms.

(iii) *The genetics of the tailed condition*

There is one feature of the Madagascan, Grand Comoro and Abyssinian races, other than the presence of the male-like pattern of the females, not found in any other race, namely the presence of tails, all being monomorphic in this respect. Now the presence or absence of tails is controlled by a pair of allelomorphs t and T whose locus is sex-controlled, autosomal and not linked to that controlling colour pattern. The allopatric tailed form of the Madagascan race produces short tails of variable length in the heterozygote with the main African stock (tables 3 and 4). The tails are further reduced in the first backcross to the African stock since in all the tailed individuals the tails never exceeded the length of the shortest ones in the F_1 hybrid (2 mm.).

In the similar hybrid using the Abyssinian race the heterozygotes are tailless in almost half of the individuals and the rest have short tails similar to the less extreme of the Madagascan hybrids, this difference between the two race-crosses being significant ($P<0.01$). Thus

the dominance of taillessness is complete in many individuals of the F_1 and in fact also in the first backcross into the Abyssinian race

TABLE 3

Types of tails and genotype

Hybrid	None (*Tt*)	Short (*Tt*)	Long (*tt*)
mer. × *afr.* . . . F_1 .	0	8	0
ant. × *afr.* . . . F_1 .	29	31	0
(*ant.* × *afr.*) F_1 × *ant.* . B_1 .	9	10	22

T is the allelomorph controlling the tailless condition. *mer.*, *ant.* and *afr.*, stand for the races *meriones* from Madagascar, *antinorii* from Abyssinia and the main African stock respectively. The distinction between short and no tails is a somewhat arbitrary one based on the appearance of the wing. Besides the 8 *meriones* F_1 hybrids there were 11 others all of which had short tails but these have not been measured since they were damaged while being used for breeding.

(table 3). The reason for the greater degree of dominance in the Abyssinian cross becomes clear when we compare the pure Madagascan

TABLE 4

Race or hybrid	Sex	Genotype	Total	Mean tail length in mm.	Sample variance
mer.	♂	ttH^vH^v	51	14·4	0·59
	♀	ttH^vH^v	29	14·8	0·88
ant.	♂	ttH^vH^v, H^v — — —	54	14·2	1·32
	♀	ttH^vH^v, H^v —	28	12·5	3·96
	♀	*tt* — —	8	9·0	5·33
afr.	♂	*T T* — —	15	14·9	1·00
	♀	*T T* — —	64	0·8	0·24
mer. × *afr.* F_1 . .	♀	TtH^v —	8	4·4	6·43
ant. × *afr.* F_1 . .	♀	$Tt\,H^v$ —	31	2·4	1·18
	♀	*Tt* — —	29	1·5	0·33
(*ant.* × *afr.*) F_1 × *ant.* B_1	♀	$Tt\,H^v$ —	9	2·1	0·61
	♂♀	*Tt* — —	10	1·6	0·53
	♀	ttH^v —	12	10·5	1·83
		tt — —	10	7·9	6·04

The abbreviations and genetic symbols are the same as in table 3 with the addition of H^v, which denotes the presence of the gene controlling the non-mimetic yellow pattern, and — which signifies the presence of one of the other allelomorphs of this series.

and Abyssinian races. On measuring the length of the tails of the Madagascan and Abyssinian females we find that the mean length of

those from Abyssinia is less than those from Madagascar, particularly the mimetic females (table 4). This would be expected since in the absence of the gene *T* for the tailless condition there will be selection for modifiers reducing the length of the tails in the mimetic females so that they conform more closely to the tailless models. These modifiers would presumably also act in the heterozygotes thus accounting for the complete dominance in some F_1 hybrids. Thoday (1959) has found that disruptive selection increases the variance of a population. It is therefore of some interest that the variance of tail length is greater in the Abyssinian females (particularly the mimics) than in the Madagascan and main African stock males as well as the Madagascan females (table 4). Now there would be no selection for the absence of tails before mimicry was evolved. But in the main African stock there must have been a considerable period of selection between the evolution of mimicry and the elimination of the tailed forms by the spread of the allelomorph for the tailless condition. During this period there would be selection for modifiers reducing tail length, as in Abyssinia today, and the continued presence of some of these in the main African stock would help to increase the dominance in the Abyssinian cross and also account for the reduced tail length in the Madagascan first backcross to the main African stock.

6. CONCLUSION

Although it is impossible to prove that dominance was absent when the allelomorphs controlling the mimicry of *P. dardanus* first arose, since these events are long past, the evidence from the race-crosses strongly supports such an hypothesis and further suggests that dominance has been evolved owing to the action of disruptive selection as suggested by Sheppard (1958). Furthermore there are also data which suggest that modifiers for the reduction of a character (tail length) can also lead to the evolution of dominance even in the absence of the character (taillessness) whose dominance is being affected. Our results are therefore in accord with the hypothesis put forward by Fisher (1928) that dominance is not a fixed attribute of a particular allelomorph but is modified by natural selection during the course of evolution.

7. SUMMARY

1. The various mimetic and non-mimetic female forms of the butterfly *Papilio dardanus* appear to be controlled by a multiple allelomorphic series or by closely linked genes whose effect is sex-controlled.

2. There is complete dominance between the sympatric forms except for a minority, 7 heterozygotes out of 21 tested, and here the forms concerned are either very rare or the heterozygotes resemble some other polymorphic form or selection for a close resemblance between model and mimic is relaxed on account of the rareness of the models.

3. Hybrids have been made between the main African races and those found in Abyssinia and Madagascar. The results suggest that hybrids between allopatric forms, in contrast to sympatric ones, much more rarely show complete dominance; there being 12 intermediate heterozygotes in 14 tested.

4. The results are in agreement with the view that, where there are two optimum phenotypes controlled in part by a pair of allelomorphs, disruptive selection will result in the heterozygote and one homozygote coming to resemble one optimum phenotype and the second homozygote coming to resemble the other.

5. In the main African races the females, unlike the monomorphic males, are tailless whereas the Madagascan and Abyssinian ones invariably have tails. Their absence is controlled by a single locus independent of that controlling colour-pattern.

6. Like the colour patterns, the absence of tails is incompletely dominant to the allopatric tailed condition. Dominance is more complete in the Abyssinian race-crosses than in those using the Madagascan form. In contrast to the monomorphic non-mimetic Madagascan females the Abyssinian females, particularly the mimetic ones, have shorter tails than the males. It is suggested that this results from the accumulation of modifiers, as the result of disruptive selection, for the reduction of tail length in the mimics and that these modifiers acting in the heterozygote increase the dominance of the tailless condition.

7. Our results strongly indicate that dominance between two unifactorially controlled characters is often absent initially but can evolve as the result of disruptive selection even in the absence of one of the two characters. The selection, however, is likely to be effective more rapidly if the populations are polymorphic for the two.

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8. REFERENCES

CLARKE, C. A., AND SHEPPARD, P. M. 1959*a*. The genetics of some mimetic forms of *Papilio dardanus*, Brown, and *Papilio glaucus* Linn. *J. Genet.*, *56*, 236-260.

CLARKE, C. A., AND SHEPPARD, P. M. 1959*b*. The genetics of *Papilio dardanus* Brown. *J. Genet.* (in press).

FISHER, R. A. 1928. The possible modification of the response of the wild type to recurrent mutations. *Amer. Nat.*, *62*, 115-126.

FORD, E. B. 1936. The genetics of *Papilio dardanus* Brown (Lep.). *Trans. Roy. Ent. Soc. Lond.*, *85*, 435-466.

(1)

(2)

(3)

(4)

(5)

(6)

Plate I

meriones and F_1 hybrids (1) *meriones* male-like female, (2) *hippocoonides*/yellow heterozygote, (3) *natalica*/yellow heterozygote, (4) *cenea*/yellow heterozygote. (5 and 6) extreme forms of *trophonius*/yellow heterozygote.

(1)

(2)

(3)

(4)

(5)

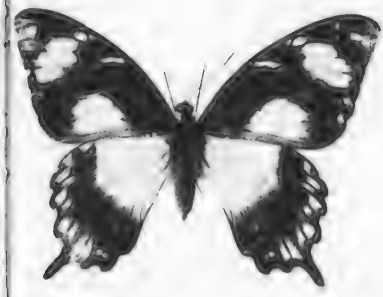
(6)

Plate II

antinorii and F_1 hybrids. (1) *antinorii* male-like female, (2) *hippocoonides*/yellow heterozygote, (3) *natalica*/yellow heterozygote, (4) *cenea*/yellow heterozygote, (5) *trophonius*/yellow heterozygote, compare with plate I, 5 and 6, (6) 2nd backcross to main African stock *hippocoonides*/yellow heterozygote. Note difference from 2.

(1)

(2)



(3)

(4)

(5)

(6)

Plate III

First backcross to *antinorii*. (1 and 2) The insects are either *hippocoonides*/yellow or *cenea*/yellow heterozygotes, (3 and 4) *hippocoonides* homozygotes (5 and 6) *cenea*/*hippocoonides* heterozygotes. Note (*a*) independent assortment of pattern and tailless condition, (*b*) the yellow *antinorii* like hindwing of 1 and 2 as compared with plate II, nos. 2 and 4, (*c*) the complete dominance of yellow over *cenea*. Compare plate II, no. 4 and plate I, no. 4.

FORD, E. B. 1953. The genetics of polymorphism in the Lepidoptera. *Advanc. Genet.*, *5*, 43-87.

FORD, E. B. 1955. Polymorphism and taxonomy. *Heredity*, *9*, 255-264.

MATHER, K. 1955. Polymorphism as an outcome of disruptive selection. *Evolution*, *9*, 52-61.

SHEPPARD, P. M. 1958. *Natural Selection and Heredity*. Hutchinson, London.

THODAY, J. M. 1959. Effects of disruptive selection. *Proc. 15th Int. Cong. Zool.*, 127-130.

VILLALOBOS, C., AND VILLALOBOS, J. 1947. *Colour Atlas*. Buenos Aires, El Ateneo.

THE MEIOTIC BEHAVIOUR, FERTILITY AND STABILITY OF WHEAT-RYE CHROMOSOME ADDITION LINES

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1. INTRODUCTION

The chromosomes of rye, *Secale cereale*, a diploid, do not pair with those of common wheat, *Triticum aestivum* a hexaploid, in normal interspecific hybrids. Since, therefore, rye genes cannot be introduced into wheat chromosomes by meiotic recombination it has been suggested that characteristics of rye might be transferred to wheat by adding whole chromosomes of rye to the full complement of wheat. This can be accomplished by sequences of backcrosses and self-pollinations in the derivatives of wheat-rye hybrids or amphiploids (Florell, 1931; O'Mara, 1940; Riley and Chapman, 1958). The most valuable derivatives of this process are disomic addition lines, which have a single pair of rye chromosomes added to the full complement of wheat, since they may be expected to be true-breeding and fertile because of their balanced chromosome number.

The purely genetical value of wheat-rye addition lines rests on the information they provide about the gene content of particular chromosomes isolated from the rest of the rye complement (Riley and Chapman, 1958). To be useful in plant breeding the rye chromosome must introduce genes which are epistatic to wheat genes or which interact with them.

However, the influence of the alien chromosome pair on fertility and meiotic regularity, and the constancy of the new chromosome status are of equal significance for the exploitation of addition lines in breeding. Moreover, since these characteristics illustrate the adequacy of the internal organisation of the genotype they present a measure of the degree to which the organisation is disrupted by the inclusion of an alien pair. Meiotic regularity, fertility and stability will, therefore, show what potential such lines would have in evolution and the extent to which the components of the genotype must be subjected to mutual adjustment for a balanced organisation to be acquired.

2. MATERIALS AND METHODS

The two species involved in the present work are *Triticum aestivum* L. emend Thell, spp. *vulgare* MacKey variety Holdfast ($2n = 42$) and *Secale cereale* L. strain. King II ($2n = 14$).

The phenotypes and origins of lines with disomic additions of rye chromosomes I, II, III and IV have been described by Riley and Chapman (1958). One further line, which had the addition of a rye chromosome, designated V, is discussed in

the present paper. This chromosome has approximately equal arms, and plants in which it is disomic differ from the phenotype of Holdfast in having a very caespitose juvenile habit. At maturity chromosome V disomics are only two-thirds the height of Holdfast and have very short dense spikes.

The system of extracting the addition lines involved only one inbred wheat variety as a parent, so the wheat chromosomes must be highly homozygous. Since, also, all the addition material stems from one hybrid plant from a wheat-rye cross, only one haploid sample of rye chromosomes is involved, so that the rye chromosomes which are disomic in the addition lines must also be homozygous, within the limits of mutation.

Meiosis was studied in orcein-Feulgen squashes of pollen mother cells from the anthers of greenhouse-grown plants. Three generations of disomic additions were sampled each in a separate season, only one generation of monosomic additions was examined. About 50 pollen mother cells per plant were scored.

In estimating the stability of the lines, the chromosome numbers of progenies were determined on squashes of root-tips pre-treated in mono-bromonaphthalene and stained by the Feulgen method. The freshly germinated grains, from which the root-tips were sampled, were from greenhouse-grown spikes which had been bagged to ensure self-pollination.

The fertility of disomic additions was estimated as the proportion of primary and secondary florets with grains. Florets were counted over the whole length of two spikes of each plant of a sample grown in the greenhouse. The spikes used had all been bagged to ensure self-pollination.

3. OBSERVATIONS

(i) *Meiotic behaviour*

First metaphase, on which the meiotic behaviour of the addition lines has been principally judged, provides a satisfactory numerical assessment of regularity. It is the earliest stage which can be easily examined in cereals and any major abnormality during this phase would be reflected in either the fertility of the parents or the genetic stability of the progeny. It offers, therefore, a key to the adequacy of the combined operation of the wheat and rye components of the lines.

In 43-chromosome monosomic additions, with a single rye chromosome transferred to the full wheat complement, 21 bivalents and one univalent would normally be expected at meiosis. Similarly in 44-chromosome disomic additions, with a balanced chromosome number —the rye chromosome being represented twice—22 bivalents would be expected.

The recipient variety, Holdfast, usually had 21 bivalents at meiosis (table 1) but in 6 per cent. of pollen mother cells the formation of one or two bivalents had failed, leaving unpaired univalents. A frequency of cells with univalents of about this order is commonly found in *T. aestivum* but the frequency differs a good deal between varieties (Thompson and Robertson, 1930; Hollingshead, 1932; Person, 1956). The persistence of such irregularity in wheat must be related to the polyploid status of the species. This provides sufficient duplication in the genotype to ensure that the monosomic zygotes which result from the functioning of gametes, particularly embryo

sacs, deficient for a chromosome are perfectly viable. The high fertility of monosomics in wheat (Sears, 1954) and the rapid return of their derivatives to the euploid condition must also temper the selection against univalent formation. For if, as Sears (1953) has demonstrated, a monosomic produces in its progeny a ratio of approximately one euploid : 3 monosomics, and assuming no differential selection, then 5 generations after the origin of a monosomic 75 per cent. of its descendants will again be euploid.

Hexaploid wheat will therefore tolerate a level of meiotic abnormality which would be a severe disadvantage in a diploid. Further evidence of this is provided by the single quadrivalents which were found very rarely in Holdfast pollen mother cells, as well as in cells of the addition lines. A similarly infrequent occurrence of quadrivalents, which are probably due to the conjugation of homoeologous (Huskins, 1931; Sears, 1956) chromosomes from different genomes, has been noticed in other wheat varieties (Ellerton, 1939; Love, 1939; Huskins, 1941).

The univalents found in wheat and also in the disomic addition lines may have resulted either from the failure of the chromosomes to pair at zygotene, asynapsis, or from paired chromosomes, separating at diplotene because chiasma formation had not occurred, desynapsis. In wheat, chromosomes which have paired at prophase without chiasma formation often remain in secondary, side-by-side, univalent associations until metaphase (Person, 1955; Riley and Chapman, 1957). However, the univalents in the present material were rarely in side-by-side associations so that they were probably asynaptic rather than desynaptic in origin. This suggests disturbances of zygotene or pre-zygotene processes rather than interference with the timing or the intimacy of pachytene association, or with the control of chiasma formation.

The univalent frequency was greater than that of Holdfast in all the additions (table 1). Cells with two univalents constituted the majority of those in which there was some pairing failure, but it was generally impossible to determine whether the univalents were rye or wheat chromosomes. The paired rye chromosomes could be recognised in occasional cells with univalents but in view of the frequency of univalents in wheat itself it is not surprising that such cells should occur. However, in most cells with two univalents it was probably the alien chromosomes which were unpaired since the frequency of cells with four univalents was rarely higher than that with two in Holdfast. Naturally in cells with four or more univalents there must have been unpaired wheat chromosomes but the data for none of the lines, except III, show any evidence of increased asynapsis of the wheat complement.

There were considerable differences between the addition lines in meiotic regularity (table 1). Disomic additions of II and V in which more than 90 per cent. of pollen mother cells were fully paired

were almost as regular as Holdfast, despite the extra pair. Nor had they any cells with more univalents than Holdfast. Moreover these two chromosomes, with chromosome I, had the least effect on the conjugation of wheat chromosomes in monosomic additions (table 2). Apparently, therefore, these rye chromosomes cause little disturbance of the regulation of meiosis in wheat, and when disomic fit harmoniously into the pattern of wheat meiosis.

TABLE 1

Meiotic chromosome pairing in disomic addition lines

Line	Plants	Cells	Proportion with bivalents only	Proportion of cells with various no. of univalents		
				2	4	6
II	10	850	0·91	0·09	...	...
III	9	470	0·71	0·21	0·06	0·02 *
IV	14	730	0·85	0·13	0·01	0·01 *
V	4	210	0·91	0·08	0·01	...
Holdfast	4	200	0·94	0·05	0·01	...

* There was one cell with ten univalents.

Monosomic additions of chromosomes III and IV showed a marked increase in the amount of asynapsis of wheat chromosomes compared with Holdfast, and it is evident that they carry genes which interfere with the pattern of wheat pairing. This is also apparent in the disomic additions in the extended range of univalents per cell, and the higher

TABLE 2

Meiotic chromosome pairing in monosomic additions

Line	Plants	Cells	Proportion of cells with various pairing		
			21″ 1′	20″ 3′	19″ 5′
I	3	150	0·90	0·09	0·01
II	3	150	0·91	0·09	...
III	3	150	0·83	0·16	0·01
IV	3	150	0·83	0·15	0·02
V	3	150	0·96	0·04	...

frequency of cells with four univalents in the chromosome III material. There were much lower proportions of cells with complete pairing in both III and IV disomic additions, mainly due to the high frequency of cells with two univalents. The two univalents were probably rye chromosomes in most cells, so that chromosomes III and IV as well as causing more wheat asynapsis were also less likely to pair themselves than were II and V.

There was no evidence of post-metaphase meiotic errors which could not be ascribed to the presence of univalents at first metaphase. Throughout sporogenesis, therefore, in some lines there were no greater irregularities than occurred in wheat, but in others there were persistent effects of the prophase asynapsis caused by disorganising interactions between the wheat and rye components.

(ii) *Fertility*

Estimates of fertility have been based on seed set because it measures the immediate efficiency of a line in terms of evolution and crop production. The number of grains set per spike was recorded, as well

TABLE 3

Fertility of disomic addition lines

Line	Plants	Fertility of primary and secondary florets	Grains per spike
II	19	0·50 ± 0·11	26·1 ± 2·4
III	18	0·02 ± 0·01	5·2 ± 3·5
IV	25	0·74 ± 0·02	56·9 ± 2·0
V	10	0·56 ± 0·03	29·4 ± 1·9
Holdfast	10	0·91 ± 0·02	48·0 ± 1·1

as the proportions of primary and secondary florets fertile, so that an estimate of the total productivity of the line should be available.

It is clear that the fertility of all the lines examined was well below that of Holdfast (table 3). There were, moreover, very pronounced differences between the lines. Disomic additions of chromosome IV

TABLE 4

Fertility of monosomic additions

Line	Plants	Fertility of primary and secondary florets	Grains per spike
I	11	0·69 ± 0·03	30·5 ± 3·2
II	9	0·64 ± 0·05	42·3 ± 4·7
III	6	0·37 ± 0·08	29·0 ± 6·4
IV	13	0·59 ± 0·04	39·0 ± 4·7
V	9	0·59 ± 0·02	32·0 ± 3·3
Holdfast	10	0·91 ± 0·02	48·0 ± 1·1

most nearly approached Holdfast, having 80 per cent. of its fertility, and because of much larger spikes, producing more grains per spike than the recipient variety. Under field conditions this would not give the IV addition line superior reproductive value because it has fewer spikes per plant and per unit area of ground. Whilst, on average, a

spike of a IV disomic addition plant produces more grains than a Holdfast spike it is much less efficient having 26 per cent. compared with 9 per cent. of sterile florets.

The extremely low fertility of disomic additions of chromosome III was mainly due to the frequent failure of the anthers to dehisce because of a reduced proportion of apparently viable pollen grains. Only about 44 per cent. of the pollen of III disomics had contents which would take up aceto-carmine stain whereas the other lines and Holdfast had over 95 per cent. stainable pollen. The low fertility may be related to the meiotic irregularity of this line. However, most meiotic disturbances must have resulted in the production of euploid pollen with twenty-one wheat chromosomes and consequently of normal viability. If the low ferility of the line cannot be ascribed to meiotic irregularities then it must be due to the genetic effects of the chromosome, a conclusion which also accords with the low fertility of the monosomic addition lines.

It may be questioned whether the genetically conditioned infertility of chromosome III additions is related to the effects of the self-incompatibility alleles of rye. However, the association of infertility with poor pollen production and non-dehiscence of the anthers does not suggest the operation of an S allele. Moreover there is no evidence of the self-incompatibility mechanism of rye in wheat-rye amphidiploids in which the entire chromosome complements of the two species are combined, so it would be surprising to find any in single pair addition lines.

Thus the two lines involving chromosomes III and IV differed in having the highest and the lowest fertility amongst the lines considered but were similar in having the most irregular meiosis. This comparison strengthens the conclusion already drawn from the chromosome III additions, that reduced fertility was of genetic rather than cytological origin. Moreover, further support may be adduced from the observation that although disomic additions with chromosomes II and V were only slightly more irregular than Holdfast at meiosis yet they had little more than half its fertility. Indeed the low fertility of the addition lines is striking evidence of the impact of the deleterious interactions of rye and wheat genes.

(iii) *Stability*

As might have been expected from their meiotic behaviour, no addition line was completely stable; all produced a small proportion of progeny which deviated from the chromosome number of the parent (table 5). Most of the deviants were 43-chromosome monosomic additions which probably resulted from the functioning of gametes, deficient for the rye chromosome, which arose from meiotic divisions in which the rye chromosomes were unpaired. No 43-chromosome plant was recorded in which the rye chromosome remained disomic

but a wheat chromosome was monosomic, although such plants could arise from cells with the rye chromosomes conjugated but with wheat univalents. Even assuming the random functioning of all classes of gametes 43-chromosome wheat monosomics would be very infrequent. However, the frequencies of plants deficient for a rye or for a wheat chromosome must have been affected by the relative competitive values of the two sorts of 21-chromosome pollen compared with the usual 22-chromosome pollen. The pollen deficient for the rye chromosome, having returned to the euploid conditions, would compete vigorously with the 22-chromosome addition pollen and so would probably have a proportion of success in fertilisation in excess of its representation in the pollen population. By contrast it may be that pollen deficient for a wheat chromosome would have less success in fertilisation than 22-chromosome pollen and so wheat monosomics

TABLE 5

Chromosome numbers of the progeny of disomic additions

Line	Plants	Proportion with various chromosome numbers				
		41	42	43	44	45
II	64	...	...	0·06	0·91	0·03
III	23	0·04	...	0·09	0·83	0·04
IV	51	...	...	0·08	0·92	...
V	61	0·01	...	0·18	0·79	0·02

would be relatively under-represented in the progeny of a disomic addition.

Very rarely 45-chromosome plants were found in the progenies of 44-chromosome disomic addition parents. These probably resulted from the functioning of 23-chromosome gametes which arose when both partners of an asynaptic pair were incorporated in the same gamete.

There was no relationship between the stability and the fertility of the lines, but stability reflected meiotic regularity, although imperfectly. The line involving chromosome III, which was the most irregular at meiosis was not unstable to the same extent, and whilst the V line had the most regular meiosis, it was the least stable.

Monosomic addition plants segregated an excess of 42-chromosome progeny (table 6). Indeed the occurrence of about 75 per cent. without the unpaired chromosome, and of about 25 per cent. with it, is consistent with the segregation of monosomic chromosomes in wheat (Sears, 1953; Morrison, 1953). This pattern of segregation in wheat arises from the transmission of the unpaired chromosome to about one-quarter of all gametes but the almost exclusive success in fertilisation of euploid pollen. By analogy, therefore, if the rye chromosomes

were included in about a quarter of the embryo sacs, 22-chromosome pollen can rarely be successful, except in the chromosome III line.

The instability observed would lead to a rapid decay of the alien disomic component of a population which was originally entirely disomic. The shift of such a population to the euploid state would be exaggerated by the excess of euploids in the progeny of 43-chromosome monosomic additions (table 6) which would quickly be established in the population. Thus, with a fresh increment of monosomic additions from disomic additions each generation, the return of most of the derivatives of monosomic additions to the euploid status and the stability of the euploids, there would be a continual run down to the euploid level and no return. Consequently, a character introduced with a rye chromosome would have to possess enormous selective

TABLE 6

Chromosome numbers of the progeny of monosomic additions

Line	Plants	Proportion with various chromosome numbers				
		41	42	43	44	45
I	219	0·01	0·77	0·20	0·02	...
II	47	0·03	0·70	0·21	0·06	...
III	115	...	0·60	0·25	0·14	0·01
IV	56	...	0·71	0·25	0·04	...
V	58	0·02	0·59	0·34	0·03	0·02

value in evolution or agriculture to outweigh its elimination from the population by the loss of the alien chromosome.

4. DISCUSSION

The meiotic behaviour, fertility and stability of the present addition lines were generally consistent with what has previously been observed in similar material. Florell (1931), whilst observing that the alien chromosomes tended either to precede the wheat chromosomes or to lag behind them at first anaphase, thought, from the cytological evidence, that constant wheat-like strains with a rye chromosome could be produced. O'Mara's (1953) conclusions, based principally on the study of rye chromosomes I, were closer to those that may be drawn from the present work, " Since the rye chromosome often occurs in the univalent condition, even when it is present as two homologues, stable additions of the rye chromosome to wheat would seem to be improbable ". However, the line with which O'Mara (1951) dealt was less regular at meiosis and less stable than any line studied in the present work.

The meiotic behaviour of O'Mara's addition of chromosome I was similar to that of the present addition of chromosome II to Holdfast

when it was first extracted as a disomic (Chapman and Riley, 1955). The high level of 90 per cent. asynapsis which was originally observed in this line has never been repeated since (table 1), and there is no clear explanation for the different behaviour of subsequent generations derived from this plant.

Hyde (1953) had no complaint about the meiotic regularity of addition lines with pairs of chromosomes of *Haynaldia villosa* transferred to wheat, except for chromosome IV which caused asynapsis of the wheat chromosomes as well as being asynaptic itself. In the disomic addition line with the chromosome of *Ægilops umbellulata* responsible for leaf-rust resistance added to wheat, Sears (1956) found a higher frequency of cells with full bivalent formation, but a lower stability amongst the progeny, than occurs in most additions with rye chromosomes. Matsumura (1952) added single chromosomes of the D genome of the hexaploid species *Triticum spelta* to the full complement of the tetraploid *T. polonicum*, and in a line with a pair of D genome chromosomes, " D diplosomic ", there was some reversion to the euploid condition, indicating meiotic irregularity.

In alien addition lines in which pairs of chromosomes of *Nicotiana glutinosa* were added to the full complement of *N. tabacum* there was apparently about 10 per cent. of pollen mother cells in which a bivalent failed (Gerstel, 1945). This is a degree of irregularity essentially similar to that in alien chromosome additions based on wheat. It may be concluded, therefore, that no alien addition line has been produced which equals the recipient species in the efficiency of its reproductive processes.

Through the action of natural selection for high reproductive values and for restricted genetic variation the genotypes of most successful species are adjusted to condition regular meiotic division and high fertility. Part of the adjustment involves the integration of the components of the genotype so that they are mutually co-ordinated to function together harmoniously to produce the optimum expression of the selected character. It is not, therefore, surprising that the added rye chromosomes should often have failed to fit neatly into the operation of the wheat genotype, for there had been no opportunity for selection to balance the contributions of two species, long isolated and presumably widely divergent in genetic structure.

The disturbance of the meiotic behaviour of the wheat chromosomes was very slight, except in additions with chromosome III, indicating considerable genetic stability of the control of meiosis in wheat. By contrast, in some additions, the rye chromosomes were often unpaired, failing in some way to match the wheat division. These chromosomes were not entirely susceptible to the wheat control of division and had no internal regulation which was effective in the wheat background.

The instability of the lines was a symptom of their meiotic irregularity and it is through stability and fertility that selection for meiotic

regularity would normally operate. However, there was no correlation between meiotic behaviour and fertility. The generally low fertility must be accepted as a further example of the deleterious interactions of rye genes with the wheat genotype (Riley and Chapman, 1958). Indeed the failure of the two genetic components of the lines to work together is particularly evident in this character. If, therefore, alien addition material were to have a successful evolutionary future, heterozygosity would be necessary to provide the genetic variation on which selection could operate to produce a new balance within the genotype. By the system of extraction the rye addition lines examined in this study must be almost completely homozygous, but Riley (1956) has described how heterozygosity could be introduced into the rye chromosomes alone, leaving the agriculturally satisfactory wheat genotype intact.

The meiotic irregularity and instability of alien additions would be exaggerated on a population scale by the rapidity of the return to the euploid condition due to the high competitive ability of euploid pollen over addition pollen (O'Mara, 1951; Hyde, 1953; Sears, 1956; Moav, 1958). The only exception, with euploid pollen at a disadvantage, concerns the *Kl* chromosome of *Nicotiana plumbaginifolia* which when added, as a monosomic, to *N. tabacum* has a lethal effect on euploid pollen, so that only addition pollen is viable (Cameron and Moav, 1956). Normally, however, the poor competitive ability of addition pollen, symptomatic of genetic unbalance, causes a vast excess of euploids in the progenies of monosomic additions, which are usually the first products of meiotic irregularity in disomic additions. Disregarding any differential reproductive capacity, which would hasten the process, the decay of the addition fraction of a population which was originally entirely disomic would be so rapid as to give alien additions little value in agriculture, even if the reduced fertility were acceptable because of other benefits. In a natural population it is apparent that severe selective elimination of euploids would be necessary for the alien addition status to be stabilised.

Consequently, as the alien chromosome pair cannot be satisfactorily added to the full complement of the recipient species, its introduction is more likely to be successful if it replaces one of the chromosome pairs of the recipient species, to form an alien chromosome substitution line. Such lines can be extracted in the derivatives of crosses between alien addition lines and monosomics of the recipient species. The substitution of rye chromosome pairs for wheat pairs has been described by Kattermann (1938), O'Mara (1947) and Riley and Bell (1959), and the replacement of a *N. tabacum* pair by a *N. glutinosa* pair by Gerstel (1943). The advantage of substitution lines should lie in their improved stability compared with additions. For any pollen deficient for the alien chromosomes, as a result of asynapsis of the alien pair, will also be deficient relative to the euploid condition. It will therefore compete less well with substitution pollen than euploid

pollen would compete with addition pollen, following a similar elimination of the alien chromosome in addition lines.

Substitution lines may, thus, minimise the effects on stability of the lack of genetic integration between the alien chromosome pair and the recipient genotype. However, some of the genetical activity of the alien pair may still fit poorly into the overall operation of the recipient genotype, although it may be that some substitutions will produce better genetical balance than others. Presumably some chromosome pairs will match genetically and will take over each other's functions efficiently, so that they will compensate more adequately than others for the deficiency in the recipient complement. It may, therefore, be necessary to test an alien chromosome pair, to be substituted, against each pair of the recipient species. As O'Mara (1953) has pointed out, 147 different substitutions of rye chromosomes for wheat chromosomes are possible. However, studies of the capacity of chromosomes to compensate for one another will test the basic similarities of diverse genetic material. Moreover, the lines developed will permit further examination of the internal organisation of the genotype and may have practical value in crop improvement.

5. SUMMARY

1. Lines with single pairs of rye chromosomes added to the full complement of wheat chromosomes were less regular at meiosis than wheat, principally because of the failure of the rye chromosomes to pair.

2. This led to the loss of the rye chromosome from some of the progeny of disomic additions, and would cause the rapid return of an addition population to the euploid state.

3. No addition line was as fertile as the recipient wheat variety, although there were big differences between lines with different rye chromosomes in fertility as well as in meiotic regularity. This is taken to imply lack of genetic balance between the wheat and rye components of the lines.

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6. REFERENCES

CAMERON, D. R., AND MOAV, R. 1956. Inheritance in *Nicotiana tabacum*. XXVII. Pollen killer, an alien genetic locus inducing abortion of microspores not carrying it. *Genetics*, *42*, 326-335.

CHAPMAN, V., AND RILEY, R. 1955. The disomic addition of rye chromosome II to wheat. *Nature, Lond.*, *175*, 1091.

ELLERTON, S. 1939. The origin and geographical distribution of *Triticum sphaerococcum* Perc. and its cytogenetical behaviour in crosses with *T. vulgare* Vill. *J. Genet.*, *38*, 307-324.

FLORELL, V. H. 1931. A cytologic study of wheat×rye hybrids and backcrosses. *J. Agric. Res.*, *42*, 341-362.

GERSTEL, D. U. 1943. Inheritance in *Nicotiana tabacum*. XVII. Cytogenetical analysis of *glutinosa*-type resistance to mosaic disease. *Genetics*, *28*, 533-536.

GERSTEL, D. U. 1945. Inheritance in *Nicotiana tabacum*. XX. The addition of *Nicotiana glutinosa* chromosomes to tobacco. *J. Hered.*, *36*, 197-206.

HOLLINGSHEAD, L. 1932. The occurrence of unpaired chromosomes in hybrids between varieties of *Triticum vulgare*. *Cytologia*, *3*, 119-141.

HUSKINS, C. L. 1931. A cytological study of Vilmorin's unfixable dwarf wheat. *J. Genet.*, *25*, 113-124.

HUSKINS, C. L. 1941. Polyploidy and mutations. *Amer. Nat.*, *75*, 329-344.

HYDE, B. B. 1953. Addition of individual *Haynaldia villosa* chromosomes to hexaploid wheat. *Amer. J. Bot.*, *40*, 174-182.

KATTERMANN, G. 1938. Über konstante halmbehaarte Stamme aus Weizenroggen-bastardierung mit $2n \times 42$ Chromosomen. *Zeits. Ind. Abst. Ver.*, *74*, 354-375.

LOVE, R. M. 1939. The role of cytology in wheat improvement. *Proc. 7th Int. Cong. Genet. Edin.*, p. 197.

MATSUMURA, S. 1952. Chromosome analysis of the Dinkel genome in the offspring of a pentaploid wheat hybrid. III. 29-chromosome D-haplosomics and their relations to nullisomics. *Cytologia*, *17*, 35-49.

MOAV, R. 1958. Inheritance in *Nicotiana tabacum*. XXIX. The relationship of residual chromosome homology to interspecific gene transfer. *Amer. Nat.*, *92*, 267-278.

MORRISON, J. W. 1953. Chromosome behaviour in wheat monosomics. *Heredity*, 7, 203-217.

O'MARA, J. G. 1940. Cytogenetic studies on Triticale. I. A method for determining the effects of individual *Secale* chromosomes on *Triticum*. *Genetics*, *25*, 401-408.

O'MARA, J. G. 1947. The substitution of a specific *Secale cereale* chromosome for a specific *Triticum vulgare* chromosome. *Genetics*, *32*, 99-100.

O'MARA, J. G. 1951. Cytogenetic studies on Triticale. II. The kinds of inter-generic chromosome addition. *Cytologia*, *16*, 225-232.

O'MARA, J. G. 1953. The cytogenetics of Triticale. *Bot. Rev.*, *19*, 587-605.

PERSON, C. 1955. An analytical study of chromosome behaviour in a wheat haploid. *Canad. J. Bot.*, *33*, 11-30.

PERSON, C. 1956. Some aspects of monosomic wheat breeding. *Canad. J. Bot.*, *34*, 60-70.

RILEY, R. 1956. Adding individual rye chromosomes to wheat. *Wheat Information Service*, *4*, 12-13.

RILEY, R., AND BELL, G. D. H. 1959. The evaluation of synthetic species. *Proceedings First Int. Wheat Genetics Symposium*. (In press.)

RILEY, R., AND CHAPMAN, V. 1957. Haploids and polyhaploids in *Aegilops* and *Triticum*. *Heredity*, *11*, 195-207.

RILEY, R., AND CHAPMAN, V. 1958. The production and phenotypes of wheat-rye chromosome addition lines. *Heredity*, *12*, 301-315.

SEARS, E. R. 1953. Nullisomic analysis in common wheat. *Amer. Nat.*, *87*, 245-252.

SEARS, E. R. 1954. The aneuploids of common wheat. *Res. Bull. Mo. Agric. Sta.*, no. 472.

SEARS, E. R. 1956. The transfer of leaf-rust resistance from *Aegilops umbellulata* to wheat. *Brookhaven Symposia in Biology*, *9*, 1-22.

THOMPSON, W., AND ROBERTSON, H. T. 1930. Cytological irregularities in hybrids between species of wheat with the same chromosome number. *Cytologia*, *1*, 252-262.

ROLE OF THE MALE PARENT IN CROSSES INVOLVING APOMICTIC *RUBUS* SPECIES

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1. PSEUDOGAMY IN APOMICTIC *RUBUS*

The polyploid species of Rubus which comprise most of the European blackberry population are apomictic, in contrast to the few diploid species, such as the two primary European species *R. ulmifolius* and *R. tomentosus*. It is essential for fruit setting in these pseudogamous apomicts that they must be pollinated with viable pollen (Darrow and Waldo, 1933; Crane and Thomas, 1940; Gustafsson, 1943).

Focke used the term *pseudogamy* in 1881 for " parthenogenetic development where pollen-tubes effect the embryo-formation without any previous fusion of egg and sperm nucleus " (Gustafsson's translation), and it will be used in this sense in this paper. In his review of pseudogamy in apomictic Rubus species, Gustafsson (1943) has stated that " pseudogamy " consists of an act of " partial fertilisation ", that is fertilisation of the endosperm nucleus but not of the eggs, and is not due solely to hormone activity. That the latter part of this statement is probably correct is supported by the failure in the present study to obtain stimulation in *R. laciniatus* after brushing the ripe stigmas with freshly prepared concentrated aqueous extract of its pollen. Pollen from the related genus Rosa is ineffectual.

Those pollinations of pseudogamous facultative apomicts which result in more or less maternal-like seedlings, and with the production of only an occasional sexual hybrid when the pollen from another species is used, are given in inverted commas in this paper. A proposed life-cycle for the apomictic tetraploid species *R. laciniatus* is given in fig. 1, and is based on the cytological observations of Kerr (1954). This cycle is concerned only with the production of the tetraploid apomictic seedlings. The chief difference between sexual and pseudogamous Rubus species lies in the establishment of the megagametophyte, usually only one gametophyte develops in the sexual whereas from two to four are formed in the pseudogamous species, but the supranumerary gametophytes disintegrate as development progresses. The relative frequency of diplospory and apospory is a specific characteristic, according to Christen (1950).

Two series of investigations have been made in an attempt to explore further the genetical and evolutionary properties of pseudogamy in Rubus. The first study was initiated to determine whether

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an apomictic progeny derived any benefit from its parent being " cross-pollinated " with pollen from a different species, rather than with its own pollen. The second study was a more detailed investigation of pseudogamy in one apomictic species, *R. laciniatus*, in which the

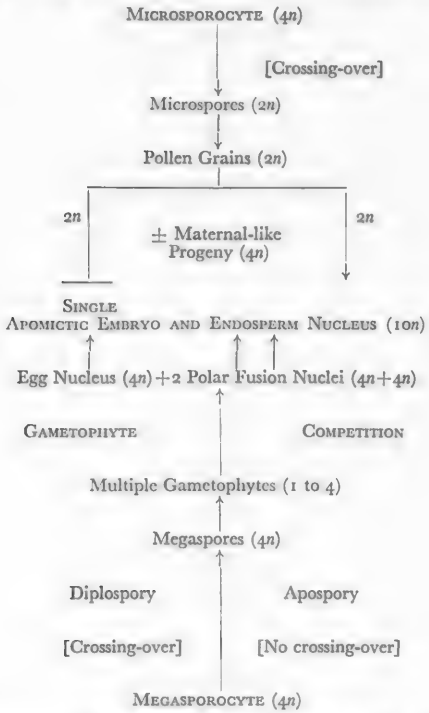

Fig. 1.—Life-cycle of a tetraploid pseudogamous apomictic Rubus plant: only the tetraploid apomictic cycle is considered. The chromosome number of the endosperm nucleus is conjectural. This diagram is based on the cytological observations of Kerr (1954) on *R. laciniatus*.

influence of different pollen parents, especially as to their effects on vigour, could be ascertained more precisely.

2. THE MALE PARENT AND VIGOUR

Individual plants of three species of cultivated apomictic tetraploid Rubi, viz. *R. procerus*, *R. laciniatus* and *R. calvatus*, were used as seed parents. Each was pollinated with its own pollen (" self-pollination ") and simultaneously some emasculated flowers were pollinated with tetraploid *R. nitidioides* (" cross-pollination "). This latter species produces about 60 per cent. good pollen grains, as measured by their staining capacity. The fruits were harvested, sown and stratified

during the winter of 1954-55; the seedlings were pricked out in the spring and later planted in the field. Heights were scored, as a measure of vigour, in the autumn when the young plants were well established, and records were made the following year of their flowering and fruiting times. The heights of the plants above or below 5 feet were also determined in the spring of 1957, when the plants were in their third year of growth. These data and the standard errors of the means are given in table 1.

The data in table 1, which are for the apomictic (*i.e.* maternal-like) seedlings only, show that the "selfed" seedlings of *R. procerus* and

TABLE 1

Vigour, flowering and fruiting in apomictic progenies of three tetraploid apomictic Rubus species, from "self-pollination" and from "cross-pollination" with R. nitidioides

Pedigree	Maximum no. in population	1st year	2nd year		3rd year
		Mean height (in.) (October 1955)	Flowering time (July 1956)	Per cent. flowering (July 1956)	Per cent. population over 5 ft. (Spring 1957)
R. procerus					
"crossed"	50	95·9±5·40 (42)	19·6±1·01 (34)	84	96
"selfed"	51	76·5±5·38 (51)	21·5±1·17 (35)	56	80
R. laciniatus					
"crossed"	50	77·8±2·01 (46)	12·2±0·89 (44)	100	94
"selfed"	50	21·6±1·35 (50)	35·1±0·55 (42)	84	76
R. calvatus					
"crossed"	8	41·3±8·18 (8)	(29·0)* ... (3)	...	(88)
"selfed"	51	57·1±4·41 (51)	22·3±0·65 (34)	66	88

Figures in brackets refer to number of plants measured; the standard errors of the means are given.

* 3 plants only; the other 5 plants did not flower.

R. laciniatus grew more slowly than those from the "cross-pollinations". By the end of the first year, the "crossed" population of *R. procerus* were, on the average, 20 inches taller than the "selfed" population. The inter-progeny difference is even greater for the two *R. laciniatus* populations, where the "crossed" and "selfed" progenies differed by 56 inches. The differences are statistically highly significant in both the species. In the *R. calvatus* progenies, however, the "crosses" were 16 inches shorter than the "selfs", but there were only eight "cross-pollinated" seedlings for comparison with the fifty "selfed", and a *t* test revealed no significant differences between the populations. Fig. 2 compares the distribution for vigour in the six progenies from "cross-pollinations" and "self-pollinations", the means of the populations being marked by arrows. The difference between the two *R. laciniatus* progenies are outstanding.

The difference in vigour between the two types of progeny from the same parent is also revealed by the difference in their flowering times *the following year*. Thus the " outcrossed " *R. laciniatus* progeny flowered twenty-three days earlier than the " selfed "; the two *R. procerus* families differed by only two days. Comparison for *R. calvatus* was not possible as only three plants of the " outcrossed " progeny were in flower.

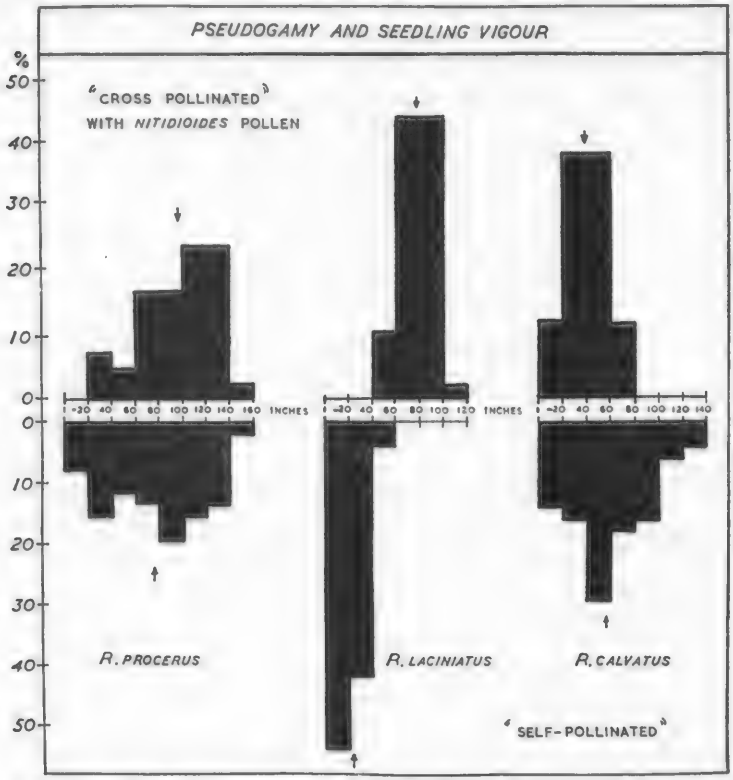

FIG. 2.—The distribution of heights among seedling progenies of 3 tetraploid apomictic Rubi that had been pollinated either with their own pollen or with that from *R. nitidioides* (4*x*). The distributions refer only to maternal-like seedlings in the " cross-pollinated " families. The arrows indicate the mean of each population.

The differences in vigour between the " crossed " and " selfed " progenies from the same parent are maintained, as seen from the proportion of plants flowering for the first time in 1956. There are clear differences for the *R. procerus* and *R. laciniatus* progenies, as shown in table 1. Such differences in vigour are continued into the third year, as illustrated by the data for heights above or below 5 feet. There was a greater proportion over 5 feet of the " outcrossed " than the " selfed "

progenies in both *R. procerus* and *R. laciniatus*; but there were no differences in the heights for the *R. calvatus* progenies. The behaviour of these *R. calvatus* progenies, which differs from those of *R. procerus* and *R. laciniatus*, will be examined later.

It was decided to use only *R. laciniatus* as the female parent for the further, more detailed studies on the effect of the male parent in view of the outstanding difference between the two progenies of *R. laciniatus*, and the ease with which true sexual hybrids are recognised in this species.

3. EFFECTS OF PSEUDOGAMY

(i) *Germination*

The pollen parent usually does not modify the number of ripe druplets developing on the berries, unless pollination is inadequate.

TABLE 2

Pseudogamous effects on germination in R. laciniatus

	Pollen parents						
	"self" (6)	2x * (1)	3x (1)	4x (7)	5x (1)	6x (2)	Mean of "cross-pollinations"
Per cent. germination by 17/4/57	2·75	30·00	0·00	7·24	7·80	14·60	11·93
S.E.	±2·43			±2·96			
Per cent. germination by 8/8/57	21·32	50·00	23·80	32·29	39·10	40·50	37·14
S.E.	±2·65			±6·52			

* = *R. ulmifolius*, a primary diploid sexual species in Europe.

Thus the mean number of druplets per fruit was 19·33 for five *R. laciniatus* plants from Fam. 41 when "self-pollinated", and the same parents had an average of 20·52 druplets per fruit following ten "cross-pollinations". Yet the role of the pollen is manifested in the following spring when the germinations commence. These effects of the pollen parent on germination in *R. laciniatus* are summarised in table 2, in which are given the germinations of six families from "selfing" and from various "cross-pollinations". The germinations by 17th April 1957, expressed as percentages of the number of seeds sown, are shown in the first row, and the total first-year germinations, also expressed as percentages of the number sown, are given in the second row.

The best germination by 17th April was in the progeny from using pollen of the common European diploid blackberry, *R. ulmifolius*. From the triploid upwards there was a steady increase in germination with increase in ploidy of the pollen parent, although there was no

germination at this stage following pollination from a triploid. The final first-year germination was also highest in the " cross " with the diploid pollen parent, and there was a regular improvement in the germination with an increase in the ploidy of the pollen parent, except for the triploid.

On the average four times as many seeds from " cross-pollination " had germinated by mid-April as from " selfed " pollinations. By the end of the first year's germination (8th August 1957) there were germinated, on the average, 37 per cent. " cross-pollinated " seedlings but only 21 per cent. from the " self-pollinations ". There are, therefore, beneficial effects on germination derived from the " cross-pollination " of *R. laciniatus*.

TABLE 3

First-year vigour of " selfed " and " crossed " apomictic seedlings from the same R. laciniatus *seed parents*

Family 41 Parental no.	Mean widths (in.) on 25th July 1957	
	" Selfed " progeny	Means of " crossed " progenies
1	7·62	7·68
6	7·62	8·20
7	9·00	23·46
10	8·43	11·65
Mean	*8·17*	*12·75*
Ratio $\frac{\text{" crossed "}}{\text{" selfed "}}$ progenies = 1·56		

These progeny differences are also seen when individual comparisons are made between pollinations of the same seed parent. For example, the highest percentage germination by 17th April (29·2 per cent.) was that from *R. laciniatus* (No. 41[7]) " cross-pollinated " by *R. magnificus* (6*x*), whereas the " selfed " seedlings from the same plant had by then germinated only 0·5 per cent. Its pollination by *R. procerus* (4*x*) gave 21·8 per cent. although by the pentaploid *R. thyrsanthus* it gave only 7·8 per cent. germination, somewhat betraying the regular increase with ploidy.

(ii) *Seedling vigour*

The difference in the vigour of young apomictic seedlings according to the type of pollination can be judged from table 3. This gives a comparison of the progenies from four seed parents of *R. laciniatus*, which had been both " self-pollinated " and " cross-pollinated " by various other species. The early seedling vigour is estimated as the

mean population width, the individual widths being measured as the greatest distance between leaf tips on 25th July 1957. There is an overall marked stimulation of the apomictic progenies from the " cross-pollinations ", and these seedlings are one and a half times as wide as those from the " self-pollinations ". I shall use the term *pseudogamous vigour* to describe the particular vigour of the maternal-like apomictic progenies.

Plate I shows the difference due to pseudogamous vigour of the apomictic seedlings of the same age amongst the progenies of one *R. laciniatus* seed parent (No. 41[7]). The two seedlings from each particular pollination were taken at random for photographing. At the top are two seedlings from " self-pollination ", the central pair are from pollination with *R. procerus* (4*x*) and the lower pair from pollination with *R. magnificus* (6*x*).

Two points emerge from this illustration. Firstly there is the extraordinary vigour of the seedlings when *R. magnificus* pollen is used, which suggests that the ploidy of the actual pollen may be important. Secondly, there is a difference according to the pollen source, as shown by the seedlings of *R. procerus* and *R. laciniatus* which have the same chromosome number. The *R. procerus* pollen produces a heterotic stimulatory effect on the pseudogamous vigour which I term " *pseudogamous heterosis* ". The differences are not to be interpreted as due simply to differences in the time of germination: these seedlings were all of the same age as determined by germination date.

Fig. 3 shows the mean seedling vigour of the apomictic progenies in eight *R. laciniatus* seed parents following various pollinations, including " selfings " and " crossings " by a polyploid series of pollen parents. The apomictic " selfed " progenies, with one exception, are close to each other in their mean seedling vigour. On the other hand, there is a wider range in vigour of the " cross-pollinations ". For one parent the two apomictic families resulting from " cross-pollination " by a tetraploid and a hexaploid pollen parent are far more vigorous than the other progenies. The progeny from one of the hexaploid pollinations, which does not show this pseudogamous vigour, involved my synthetic F_1 hexaploid hybrid of *R. laciniatus* × *R. nitidioides*. As this pollen parent probably contains four doses of *R. laciniatus*, it might be expected to give a progeny with vigour not dissimilar from " self-pollination ".

(iii) *Seed shape*

Seed shape is influenced by various internal and external factors. Theoretically, when both the embryo and the endosperm are sexually formed, the xenia might be expected to show itself as differences in seed sizes and shapes. Where the embryos arise apomictically, and so are genetically similar, they will tend to have the same shape. Modifications will, however, arise from the influence of the sexually formed endosperm, and from whether the embryo has arisen from apospory or

diplospory. Polyhaploid embryos will be expected to produce smaller seeds outside the range of variation of the apomictic seeds. Hence variation in seed shape in an apomict may be informative in revealing the effect of the pollen parent.

As there were differences in the germination and in the vigour of the seedlings according to the pollen parent used, it was decided to

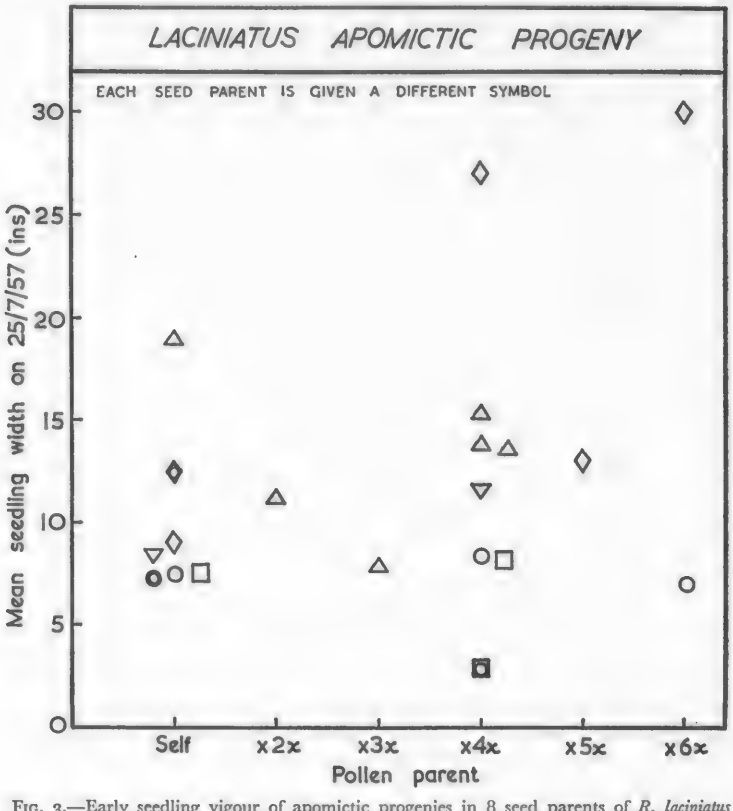

FIG. 3.—Early seedling vigour of apomictic progenies in 8 seed parents of *R. laciniatus* from either " self-pollination " or " cross-pollination " by a polyploid series of pollen parents.

test whether the pollen had an effect on the form and size of the seeds. If pseudogamy in Rubus were to act through the endosperm, as a result of endosperm fertilisation, differences in the seed pattern according to the pollen used would help to indicate if it had a prime influence prior to germination and emergence. Kerr (1954) has already observed in *R. laciniatus* that " cross-pollinations " produced larger embryos and greater endosperm development than " self-pollinations ".

One plant of *R. laciniatus* (No. 41[43]) was pollinated with four types of pollen, and the lengths and breadths of fifty seeds determined from each of the four pollinations. There are highly significant correlations between the lengths and breadths of seeds from the pollination by *R. nitidioides* (4*x*), *R. fragrans* (6*x*) and *R. dasyphylloides* (7*x*), showing a high degree of apomixis and the homeostatic effect of hybrid endosperm, but a correlation is not evident for the seeds obtained for " selfing ". The seeds from these " cross-pollinations " are, on the average, longer (73·3: 69·0 in units) and broader (57·6: 51·0) than from the " self-pollinations ". In terms of their ranges, the " selfed " seeds are much more variable in length than the " cross-pollinated " ones (*i.e.* ranges of 41 and 23 in unit differences respectively), although there is no change in the ranges of their breadths (ranges of 20·7 to 20·0 respectively).

The dimensions of seeds from various *R. laciniatus* mother plants following various pollinations were also determined, and the correlation coefficients between the lengths and breadths of seeds in various " selfed " and " crossed " progenies from different mother plants of *R. laciniatus* were calculated. The values for *r* in three of the seed parents are lower in the " selfed " progenies, which means that there is greater variation in seed shape from using the pollen of *R. laciniatus* itself. These data may be interpreted as providing some evidence that pseudogamy in Rubus involves at least a genetic change in the endosperm. The increased variation that sometimes results from " self-pollination " represents the segregation between sexually formed endosperms, as would be expected from inbreeding a heterozygous amphimictic parent.

4. PARENTAL TAXONOMY AND SEXUALITY

Gustafsson (1943) has supported Lidforss' conclusion that the frequency of sexual hybrids in facultative apomicts is not proportional to the relationship of the parents. As this conclusion, according to Gustafsson, has at times been misinterpreted, it was re-tested by using Watson's (1958) classification of the genus. The frequency was recorded with which hybrids occurred when *R. laciniatus* was pollinated by eleven other species ranging from Sylvatici to Glandulosi, and its tetraploid and hexaploid hybrids with *R. nitidioides*. The occasional sexual hybrids were readily recognised by their non-laciniate leaves, lighter green stems and differences in their prickle shapes.

The data support Lidforss' original conclusions, *viz.* that the proportion of sexual hybrids to maternals is not related to the taxonomic affinities of the seed and pollen parents. It is possible that morphologically identical plants of *R. laciniatus* produce different amounts of sexual hybrids with the same pollen parents; but this remains to be tested. The data also emphasise that there is both pseudogamy and true sexual cross-compatibility between facultative

Rubus apomicts, regardless of the section of Eubati to which they belong. It is still possible for sexual hybridisation in these apomicts to function throughout the taxonomic gamut of the genus. The taxonomic sections of Rubus must be considered, therefore, as arbitrary aids for studying this highly polymorphic genus, and not as absolutely distinct groups.

5. APOMICTIC AND SEXUAL VIGOUR

The differences in vigour between the apomictic and the sexual seedlings from the same *R. laciniatus* seed parents were tested by comparing the mean widths of the apomictic laciniate seedlings (measured in pots on 25th July 1957) with the corresponding mean widths of the sexual seedlings from the pollinations with different species as the male parents. The means of the apomicts are from measurements of more seedlings than for the sexuals.

Even though the vigour of the apomictic progenies from one seed parent will vary according to the pollen parent, their vigour, on the average, is greater than for the corresponding sexual seedlings; it may even be considerably greater. Although one might expect the sexual seedlings to show hybrid vigour, generally the apomictic seedling populations are more likely to have a natural advantage over the sexual seedlings. This will be especially valuable where local seedling competition is intense, with the maternal progenies benefiting through the pseudogamous vigour that results from " cross-pollination " between Rubus species. The few sexual seedlings produced would thus be at a competitive disadvantage to the vigorous apomicts, closed populations of which are maintained more or less undisturbed by the crowding-out of these sexuals.

6. THE MALE PARENT IN PSEUDOGAMOUS SPECIES

All apomictic species of Rubus must be pollinated with viable Rubus pollen for fruit formation. As most wild and cultivated Rubi are polyploid and pseudogamous, it might not be unexpected, therefore, that as a result of natural selection the genetic constitutions of the male gametes have considerable influence on the vigour and survival of the apomictic seedlings.

There is an increase in the vigour of the progenies after " cross-pollination ", although this need not necessarily be so. Lasheen and Blackhurst (1956) have shown that endosperm is present in Rubus seeds, and as pollination is a prerequisite of seed setting, it might be that sexually formed endosperms influence the vigour of the non-sexual embryos in the apomicts. Seeds of *R. laciniatus* " crossed " with *R. magnificus* would have 11*n* endosperm, but both the " selfed " seeds and those from crosses with *R. procerus* would have 10*n* endosperm; hence both genotype and ploidy of the endosperms are important.

The analyses of seed variation provide support for the hypothesis that the endosperm is, at least partly, responsible for the manifestation of pseudogamous vigour.

If the pollen played no part in seed formation, the scattering on a graph of the seeds from any one mother plant should be the same regardless of whether this had been " self-pollinated " or " cross-pollinated ". This is not, however, borne out by the experimental evidence, as for example, in the *R. laciniatus* plant No. 41^3, which was " self-pollinated " and also " cross-pollinated " by *R. cæsius* and *R. calvatus* respectively. There were highly significant correlations for lengths and breadths of the seeds. The mean square for the length data of the " selfed " population compared with those of the " cross-pollinated " pollinations, gives a variance ratio (V.R.) of 2·13 ($P = 0{\cdot}01$) and 1·33 ($P = 0{\cdot}05$) respectively. Similarly for the breadth measurements, the ratio of the mean square of the " selfed " population to those from pollination with " *R. cæsius* " gave V.R. = 1·99 ($P = 0{\cdot}01$) and with " *R. calvatus* " V.R. = 1·62 ($P = 0{\cdot}01$). This means that the seeds produced by " self-pollination " are significantly more variable than those from the " cross-pollinations " both in length and breadth. Similarly for plant No. 41^{43}, the data for lengths for the " selfed " population gives variance ratios of 1·97, 2·74 and 2·31 (all significant at the 1 per cent. level) for the " selfed " population as tested against " crosses " by *R. nitidioides*, *R. fragrans* and *R. dasyphylloides* respectively; but only in the " cross " by *R. dasyphylloides* is the variance ratio for breadth significant, and then only at the 5 per cent. level. The seed population means for " selfing " and " crossing " themselves show no relationship between size and pollination system, but amongst the seeds from the " cross-pollinations " the smallest means are from using $2x$*R. ulmifolius* and the largest from $6x$ and $7x$ pollen parents. The population from " cross-pollination " by *R. cæsius* is more restricted about the mean in plant No. 41^3 than that from " selfing ".

This developmental homeostatic effect conferred on seedlings by their endosperm hybridity is a further important contribution from pseudogamy to the survival of apomictic forms. The endosperms in the seeds of *R. laciniatus* from " selfing " are sexually self-fertilised, and so provide greater homeostatic instability, as manifested by the wider range and greater scatter (*cf.* Lerner, 1954). On the other hand, the endosperms in seeds from the *R. cæsius* pollinations are heterozygous, and so are more stabilised; hence the smaller scatter for seed shape.

The evidence is, therefore, that the genetic constitution of the male gamete may have considerable influence on the gametophyte competition and on vigour and survival of the maternal-like seedlings produced by apomictic Rubi. The variation in vigour between the progenies will depend, at least partly, on the hybridity of the endosperms, while the variation within the progenies is due partly to diplospory and partly to differences between the sexually formed

endosperms. The progenies of maternal-like seedlings from " self-pollination " may be expected to show less homeostatic stability than those from " cross-pollination ". The data for heights in the initial experiment (table 1) also provide support for this hypothesis. The coefficients of variation for the *R. procerus* progenies " selfed " and " crossed " by *R. nitidioides* are 50·2 and 36·5 respectively, and for the *R. laciniatus* progenies 44·4 and 17·4 respectively. Neither of these maternal parents occur in the wild, other than as escapes. The progenies of *R. calvatus* " selfed " and " cross-pollinated " by *R. nitidioides* on the other hand, give coefficients of 55·2 and 55·5 respectively. This indicates a close relationship, which is also reflected in the lack of differences between the means, and indeed Watson (1958) has placed them close together in the section Sylvatici.

Possibly in natural conditions symbiotic relationships have been established between apomictic Rubus species, which form the great bulk of the genus (Haskell, 1955). There may be as much as 20 per cent. outcrossing and this would provide an opportunity for producing those maternal-like seedlings with the benefit of pseudogamous heterosis. They would have enhanced vigour, better germination and improved powers of competition; they would also be more likely to reproduce a year earlier than the " selfed " seedlings, although not all the cross-pollinations would be advantageous.

Pseudogamy in Rubus can now be pictured as a " twilight " stage in a downward evolutionary path of sexual behaviour, between complete sexuality, involving fertilisation of egg and central fusion nucleus, and complete non-sexuality. The genetic constitution of the sexually formed endosperm in these pseudogamous apomicts affects the vigour of the apomictic offspring, often leading to pseudogamous heterosis, or luxuriance. The further one investigates the breeding behaviour of this highly successful genus, more facets are revealed to what Crane (1940) has called its " reproductive versatility ".

7. SUMMARY

Three pseudogamous Rubus species were pollinated by their own pollen, and by pollen from *R. nitidioides*. Two of the apomictic progenies from self-pollination were weaker than the corresponding pollinations by *R. nitidioides*. This was also reflected the following year in their later flowering and in the proportion that flowered.

Pseudogamous *R. laciniatus* was pollinated by a polyploid series of apomictic species. There was an improvement in early germination and in first-year germination with increase in the ploidy of the pollen parent; but pollination by the sexual diploid *R. ulmifolius* gave the most vigorous germinations.

Generally there was marked stimulation of the *pseudogamous vigour* of the apomictic progenies of *R. laciniatus* from pollinations with other species. This is termed *pseudogamous heterosis*. It varies with the

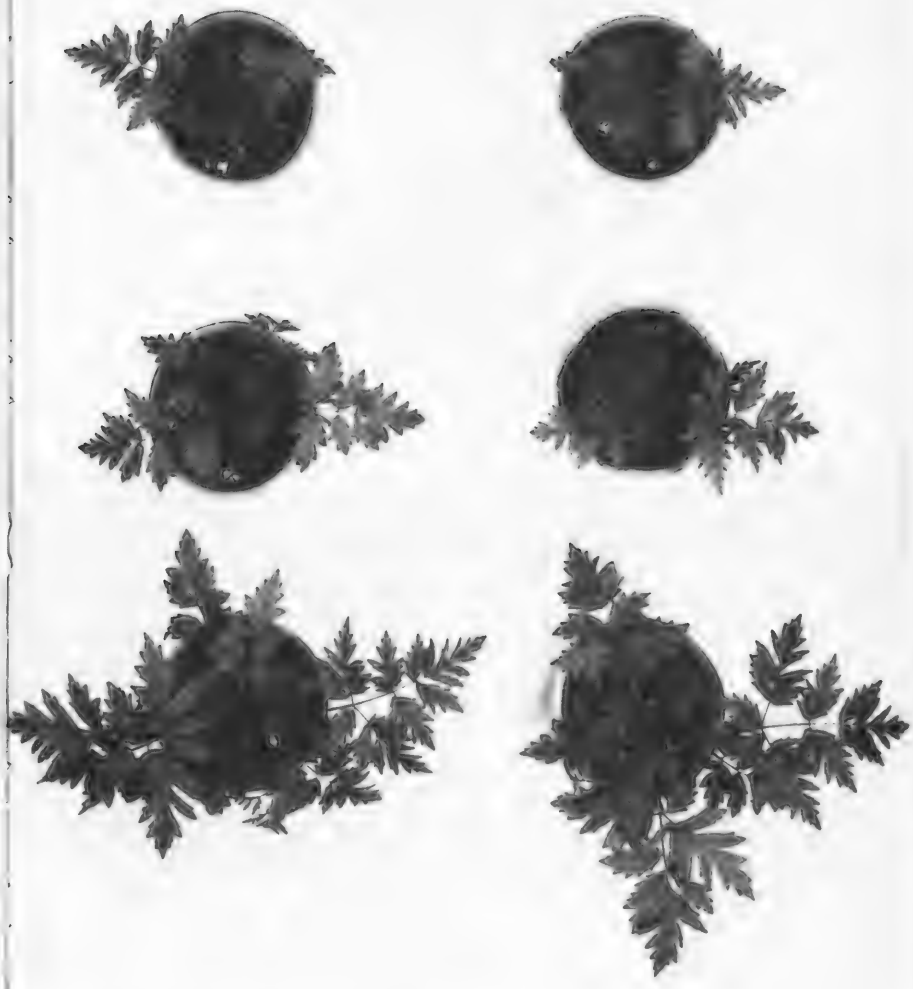

Plate I

Pseudogamous vigour in apomictic seedlings of the same age from one seed parent of *R. laciniatus*. Paired seedlings at top from "self-pollination"; centre, from "cross-pollination" with *R. procerus* (4x); bottom, from "cross-pollination" with *R. magnificus* (6x).

pollen source, and is affected by the degree of ploidy and genetic differences between the pollen parents.

The pollen parent affects seed shape in *R. laciniatus*. Lengths and breadths of seeds were correlated for most cross-pollinations, but self-pollination may reduce the correlation through increasing the variation in seed shape, particularly their lengths. This could be related to the sexuality of the endosperm in pseudogamous Rubi.

The occurrence of sexuality in *R. laciniatus* was tested by pollinations with British species of known taxonomic position. Lidforss' conclusion was confirmed that the frequency of sexual seedlings is not related to the relative taxonomy of the parents. The sexual hybrids are generally weaker than the apomicts.

8. REFERENCES

CHRISTEN, H. R. 1950. Untersuchungen über' die Embryologie pseudogamer und sexueller Rubusarten. *Ber. Schweiz. Bot. Ges., 60,* 153-198.

CRANE, M. B., AND THOMAS, P. T. 1940. Reproductive versatility in *Rubus*. *J. Genet., 40,* 109-128.

DARROW, G. M., AND WALDO, G. F. 1933. Pseudogamy in blackberry crosses. *J. Hered., 24,* 313-315.

GUSTAFSSON, Å. 1943. *The Genesis of the European Blackberry Flora.* Lund.

GUSTAFSSON, Å. 1945. *Apomixis in Higher Plants.* Lund.

HASKELL, G. 1955. Analyses of sexual-apomictic blackberry populations and their ecological consequences. *Cold Spring Harbor Symp. Quant. Biol., 20,* 111-126.

KERR, E. A. 1954. Seed development in blackberries. *Can. J. Bot., 32,* 654-672.

LASHEEN, A. M., AND BLACKHURST, H. T. 1956. Biochemical changes associated with dormancy and after ripening of blackberry seed. *Proc. Amer. Soc. hort. Sci., 67,* 331-340.

LERNER, I. M. 1954. *Genetic Homeostasis.* Edinburgh.

WATSON, W. C. R. 1958. *Rubi of Great Britain and Ireland.* Cambridge.

SEASONAL VARIATION IN LIMA BEAN SEED SIZE: AN EXAMPLE OF GENOTYPIC-ENVIRONMENTAL INTERACTION

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1. INTRODUCTION

It is a common observation that identical genotypes show drastic differences in phenotypic expression of quantitative characters when an organism is grown under widely different environmental conditions. More interesting to the evolutionist and breeder are such conspicuous fluctuations in phenotypic expression within apparently homogeneous environments. Genotypic-environmental interactions of this latter type, which presumably result from subtle but nevertheless important variations in micro-environment, have received increasing attention in recent years, both experimentally and analytically. See, for example, discussions by Comstock (1955), Dempster (1955), Lerner (1958), and Mather (1955). The present paper considers some variations in seed size observed in populations of lima beans, *Phaseolus lunatus* L., that were grown in an apparently constant environment.

2. METHODS AND MATERIALS

The populations studied were derived from hybrids between homozygous parental lines that differed in allelic constitution at a number of loci governing conspicuous morphological or colour differences. Each year a population of about 2500 plants was grown for each hybrid. Mass segregation began in the F_2 generation and continued until the F_7, F_8, or F_9 generation. Seeding rates, which were those used in commercial practice, produced stands of about 5 plants per foot in rows 30 inches apart. Each year the entire plot of each population was harvested, and a random sample of seed was set aside to establish a plot the next year. No conscious selection was practised at any time. The frequency of various genotypes in each population from year to year was based on a random sample of 4000 to 8000 seeds from the total harvest of about 100,000 seeds produced in each population in each year. Mean seed weight, expressed in grams per 100 seeds, was recorded for each genotype. This sample was found to be adequate to reduce to very low levels any random fluctuations in genotypic frequencies, and in seed size for each genotype. On the basis of previous experience, the standard error of the mean seed size would be substantially smaller than $\frac{1}{2}$ grm. per 100 seeds. Seed sizes as expressed in the graphs will therefore be within 1 grm. of the population value ($P<0{\cdot}05$).

The plots were irrigated regularly to keep soil moisture well above wilting point, and were weeded but not thinned. Other factors of the environment, such as the date of planting and harvesting, were also controlled. Within any year, the total area of the plot containing all the populations was relatively small so ensuring relatively constant soil conditions. The position of the plot was moved each year, so that some between-year fertility differences could arise, but such effects are thought to be unimportant. Since the summer climate in California is relatively uniform from year to year, the environmental conditions under which the experiment was conducted were as uniform as is likely to be encountered outdoors.

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The genes mentioned are :

cc—white seed coat irrespective of the genotype at any other locus.

C—red seed coat. This is a basic colour factor and must be present for the expression of genes *P* and *R*.

P—purple seed coat, hypocotyl and flower in the presence of *C*.

R—dark-red seed coat and red hypocotyl in the presence of *C*.

S—changes red, dark-red or purple seed coat to red/buff, dark-red/buff, or purple/buff respectively. The heterozygote *Ss* produces a diffuse type of mottling, and *SS* a restricted mottling.

The populations were made up from the following crosses :

Population no.	Cross	Genotype
53	Wilbur × L48	*ccssppRR* × *CCSSPPRR*
57	Green-seeded Henderson × L124	*ccssppRR* × *CCssppRR*
59	Wilbur × L121	*ccssppRR* × *CCSSPPRR*
60	Green-seeded Henderson × L76	*ccssppRR* × *CCssPPrr*
61	Wilbur × L76	*ccssppRR* × *CCssPPrr*
65	Green-seeded Henderson × L106	*ccssppRR* × *CCSSppRR*

3. RESULTS

(I) *Interaction between populations and seasons*

In figs. 1-6, the mean seed size of various easily recognisable genotypes is plotted against the year over a period of 5 to 7 years, comprising the F_2 to F_8 generations. The genetic units under consideration here are undoubtedly not the seed-coat colour alleles alone, but segments of chromosome marked by the seed-coat colour alleles. These units would be expected to vary in size from generation to generation, as a result of crossing-over and other factors considered by Fisher (1949). Henceforth, these segments are herein identified by the Mendelian symbols of the seed-coat colour alleles by which they are identified, but implicit in this method of identification is the notion that the genetic unit under consideration is a linked block of genes. The term " genotype " is thus used in this special sense. The first three of the six graphs (figs. 1-3) are for the same set of genotypes: *CSS*, *CSs*, *Css*, and *cc*.

In population 65 (fig. 1), all four recognisable genotypes had a low mean seed size in 1952. Mean seed size reached a maximum in 1953, dropped in 1954 and 1955, rose slightly in 1956, and again in 1957. All genotypes in this population behaved similarly in each year. This was also true for population 59 (fig. 2). However, there was no correspondence between the two populations in the peaks and depressions of seed size. For example, population 59 reached a peak in 1956, a year in which the seed size of population 65 was near the minimum. Population 53 (fig. 3) presents additional evidence that behaviour differs

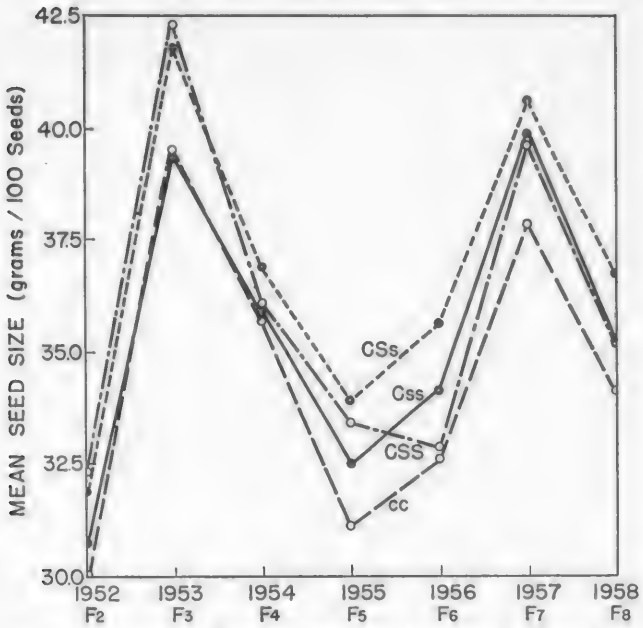

FIG. 1.—Population 65. Mean seed size of various genotypes in different years.

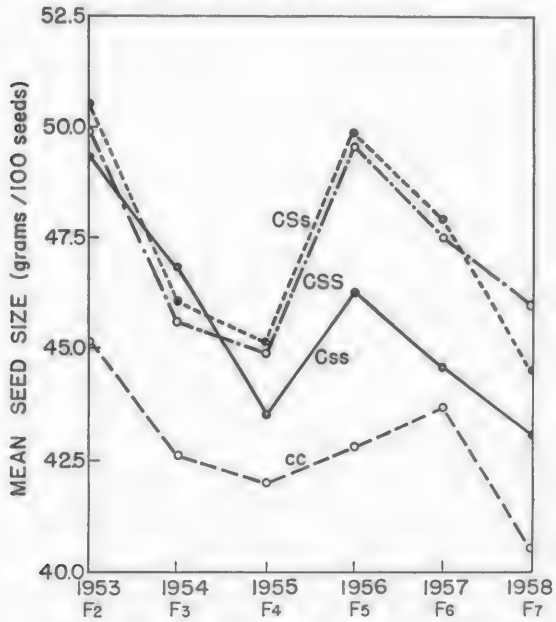

FIG. 2.—Population 59. Mean seed size of various genotypes in different years.

for each of the same four genotypes from year to year. In this population all genotypes again behaved similarly in any one year, but within-year variability among genotypes tended to be greater than in populations 65 and 59.

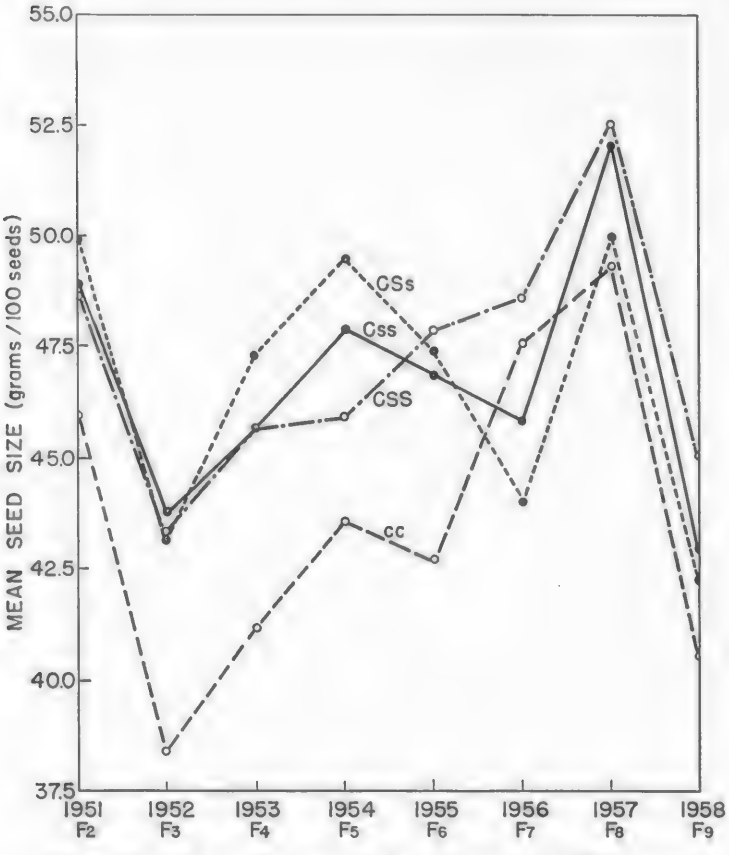

FIG. 3.—Population 53. Mean seed size of various genotypes in different years.

A number of other genotypes were also studied in the same three populations. The pattern of reaction was similar to that of the genotypes plotted in figs. 1-3. Apparently the reaction of each population to each season is unique, and the behaviour of one set of genotypes in one genetic background will not predict the behaviour of the same set of genotypes in another genetic background.

Three other populations (figs. 4-6) gave similar results. Seed size is plotted for the genotypes *CR*, *Crr*, and *cc* in population 57 (fig. 4)

and for the genotypes *CP*, *Cpp*, and *cc* in populations 60 and 61 (respectively figs. 5 and 6). It will again be noted that maxima and minima of seed size for different populations occur in different years. For example, population 57 reached a minimum in 1953, population 60 in 1956, and population 61 in 1955. The behaviour of a genotype obviously depends on the genetic background and the year. As the conditions under which the plants were grown were controlled as much

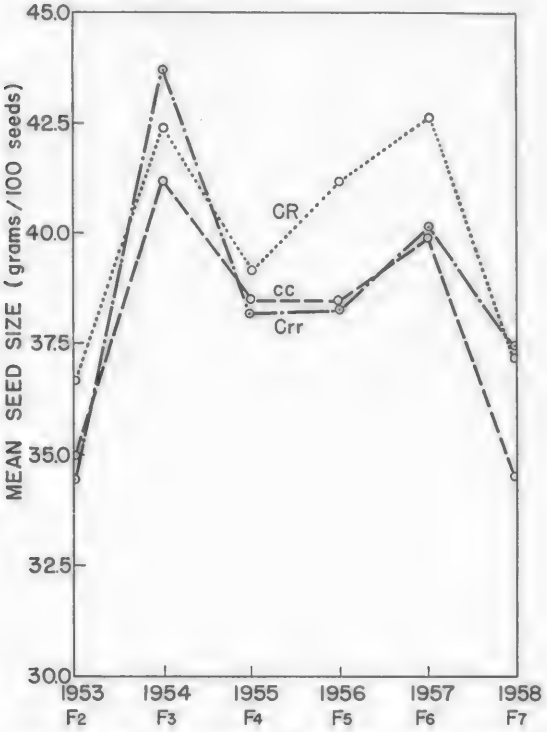

FIG. 4.—Population 57. Mean seed size of various genotypes in different years.

as possible, it is likely that the major part of this effect depends on subtle yearly climatic variations.

(ii) *Seed size of individual genotypes*

In most of the populations, seed size for the *cc* genotype was smaller than for the remainder of the genotypes. The main exception was in population 60 (fig. 5), in which mean seed size was, on the whole, smaller for the *Cpp* than for the *cc* genotype. There were other minor exceptions but usually in only one year (see figs. 1, 3, 4 and 6). The data do not reveal the portion of these differences that is due to the

pleiotropic effects of the *Cc* locus itself, and the portion attributable to seed-size genes linked with *C* or *c* in each population. Evidence from some lines differing at the *C* locus but otherwise isogenic suggests that the *Cc* locus or a very closely linked segment has a substantial effect on seed size. Figs. 4, 5 and 6 show that purple and

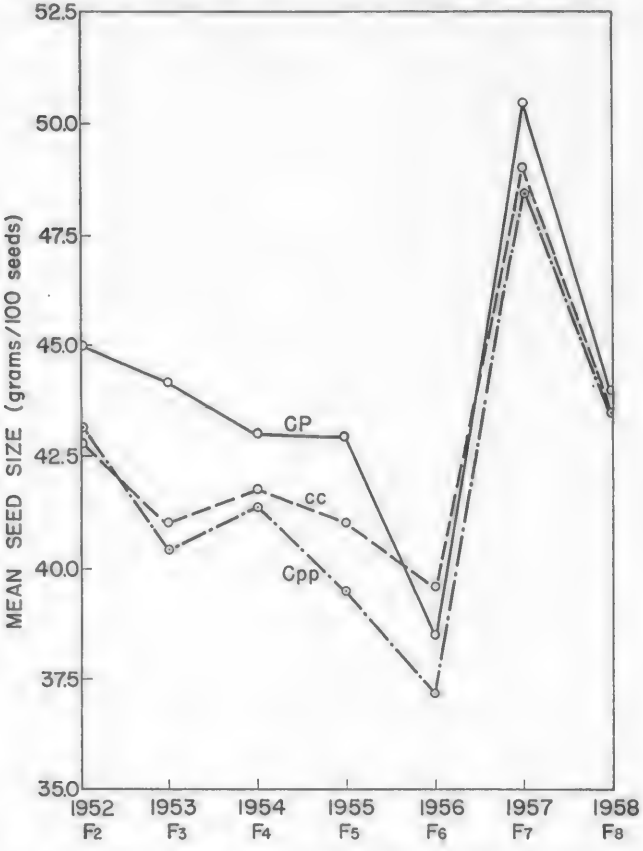

FIG. 5.—Population 60. Mean seed size of various genotypes in different years.

dark red phenotypic classes usually had a larger mean seed size than did the light red phenotypic class. In population 61 (fig. 6), *CP* had the largest mean seed size, *Cpp* was somewhat smaller, and *cc* was smallest. Thus, *C* and *P* both appear to contribute something towards the greater seed size of *CP* over *cc*. In population 60 (fig. 5), however, where *Cpp* is somewhat smaller than *cc*, only *P* appears to contribute towards the increased seed size.

In population 59 (fig. 2) in which the heterozygote, *Ss*, can be identified, the mean seed size of the genotype *CSs* was larger than that of either *CSS* or *Css*, except in 1954 and 1958. In population 65 (fig. 1), *CSs* had the largest mean seed size, except in 1952 and 1953. With population 53 it is difficult to reach a definite conclusion because

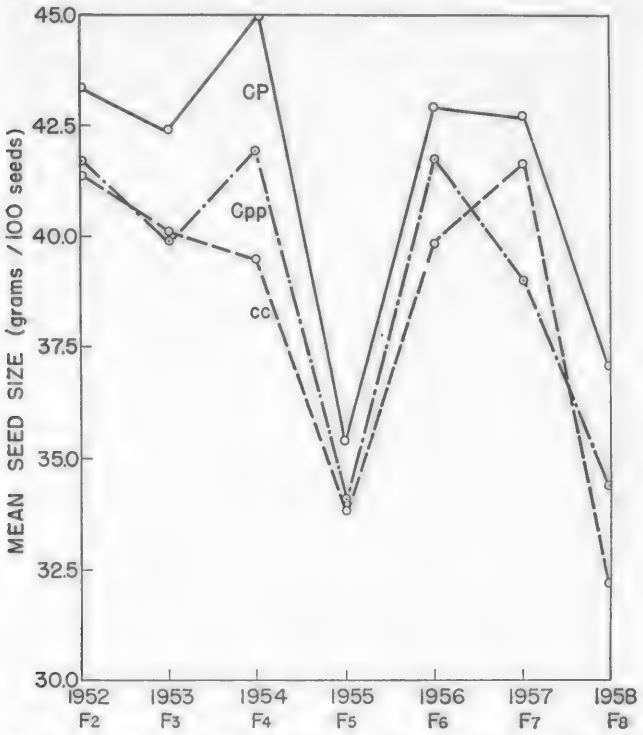

FIG. 6.—Population 61. Mean seed size of various genotypes in different years.

the mean seed size varied more than in the other populations. In general, the evidence suggests heterosis for large seed size for the chromosome section marked by the *S-s* locus.

4. DISCUSSION

The principal results of the present study can be summarised as follows: (1) seed size fluctuated sharply from year to year in each of the six populations studied; (2) these variations occurred despite the apparent stability of environmental conditions over seven years; (3) each population appeared to react uniquely to annual changes; (4) all genotypes within a population tended to react similarly in each

year. However, an occasional change in reaction patterns between years indicates that interactions did occur between specific genotypes and environment. In general, these specific interactions were slighter than the interactions between population genotypes and seasons.

These results suggest that the overall variation in mean seed size is largely determined by a genetic background differing with population. Particular genotypes within a population may differ characteristically in mean seed size, but such differences tend to remain constant from environment to environment. Thus, the population genotype in large measure determines the entire reaction system and it is the main controlling force in determining response to environmental changes, including the response of any particular genotype within the system.

The consistent behaviour of genotypes within a population and year occurred despite considerable changes in population structure as the experiment progressed. Allard and Parsons (in press) showed that self-pollination of lima beans varies from about 90 to 97 per cent. and that homozygosity increases between the F_2 and F_8 generations approximately according to the model proposed by Hayman (1953). Furthermore, selective differences between alternative alleles at many loci undoubtedly led to substantial changes in gene frequency. The frequency of *C*, for example, was about 0·5 in the F_2 generation of each of the six populations, but by the F_6 to F_8 generations had increased to 0·8 or even higher, with a concomitant decrease in the frequency of *c*.

Although the present study demonstrates that striking genotypic-environmental interactions characterise seed size in lima beans, previous studies have shown that seed size was stable for three inbred populations over four seasons (Allard, 1956). However, this result was obtained when interplant competition had been minimised or eliminated by very low planting rates. Seed size in lima beans is apparently a very complex character, depending on an intricate system of interactions between genotype and environment.

Evidence obtained from some other populations of lima beans suggests that genotypes with medium seed size (index about 40 grm./100 seeds) have higher survival value than genotypes with large (index >60) or small (index <30) seeds. Thus seed size appears to be one of the components of fitness in the lima bean. Seasonal fluctuations of seed size, though apparently fortuitous in the populations under consideration, may therefore be a mechanism contributing to the genetic variance for fitness in populations of lima beans.

Dempster (1955) discussed theoretical models in which selection pressures fluctuate from generation to generation. Variable selection pressures over a number of generations operating in conjunction with an average degree of overdominance (as at the *Ss* locus) could result in an additive genetic variance within years. Over many years, however, most of the genetic variance in such a system would result from a genotype-year interaction. A similar system may exist in lima bean

populations, indicating a need to study populations over many generations and years if predictions of possible progress under selection are to be reliable.

5. SUMMARY

Striking annual variations in seed size were shown by six populations of lima beans grown under optimal conditions in an apparently stable climate. The response of each population to each year was unique, indicating that the genotype of each population is affected differently by subtle environmental influences occurring in differing years. Specific " genotypes ", as identified by marker genes conditioning easily recognisable seed-coat colour differences, tended to behave similarly within each population. Occasional inconsistencies indicated that interactions with environment occurred between different " genotypes " within the same population.

The seeding rates used produced moderate competition between plants within a population. However, when competition between plants is eliminated by wide spacing, seed size remains constant over years; *i.e.* genotypic-environmental interactions do not occur. Thus, even under an apparently constant environment, seed size in lima beans is a complex character made up of complicated interactions between genotype and micro-environment.

There is some evidence that seed size is one of the components of fitness in lima beans. The apparently fortuitous seasonal fluctuations in seed size observed in this study may therefore be a mechanism contributing to genetic variance for fitness in populations of this species.

6. REFERENCES

ALLARD, R. W. 1956. Estimation of prepotency from lima bean diallel cross data. *Agron. J.*, *48*, 537-543.

ALLARD, R. W., AND PARSONS, P. A. Changes in gene and genotype frequencies in populations of the lima bean (in press).

COMSTOCK, R. E. 1955. The theory of quantitative genetics : synthesis. *Cold Spring Harbor Symp. on Quant. Biol.*, *20*, 93-102.

DEMPSTER, E. 1955. Maintenance of genetic heterogeneity. *Cold Spring Harbor Symp. Quant. Biol.*, *20*, 25-32.

FISHER, R. A. 1949. *The Theory of Inbreeding*. Oliver and Boyd, Edinburgh.

HAYMAN, B. I. 1953. Mixed selfing and random mating when homozygotes are at a disadvantage. *Heredity*, *7*, 185-192.

LERNER, I. M. 1958. *The Genetic Basis of Selection*. John Wiley & Sons, New York.

MATHER, K. 1955. Response to selection. *Cold Spring Harbor Symp. Quant. Biol.*, *20*, 158-165.

DIFFERENTIATION OF THE NUCLEI OF POLLEN GRAINS

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1. INTRODUCTION

THE hypothesis which is most frequently used to explain how two daughter cells become different is that, although chromosomal components are distributed equally at mitosis, cytoplasmic components may not be; differentiation must result, ultimately, from an initial cytoplasmic difference. This is shown, for example, by the differentiation of the nuclei in the pollen grains of angiosperms. La Cour (1949) found that there was an unequal distribution of ribonucleoprotein (RNP). After mitosis, one daughter nucleus, that which arrived in the area rich in RNP, became the large, diffuse vegetative nucleus. The other arrived in an area with little RNP and became the small, dense generative nucleus. La Cour concluded that nuclear differentiation was a response to cytoplasmic differentiation.

On the other hand, King and Briggs (1955), who studied developing frog embryos, found that nuclei showed stable changes in their capacity to promote differentiation. In this case, nuclear differentiation was a cause of further differentiation.

The general hypothesis with which the present study commenced was that if a difference between daughter nuclei could be demonstrated at telophase of mitosis, *i.e.* before differentiation had commenced, then the difference might reasonably be thought to be a cause of subsequent differentiation. Such a difference would not usually be expected in chromosome number nor in DNA content. The nuclear component chosen for study was basic protein because this had been shown to vary from tissue to tissue (Cruft *et al.*, 1954). Moreover, quantitative microphotometric estimation was practicable, Alfert and Geschwind (1953) having proposed a method of staining which was thought to be specific for histones or, more accurately, basic proteins with high isoelectric points.

If an unequal distribution of basic protein causes differentiation, it would not be expected at mitoses giving rise to a uniform tissue. The first mitosis in the pollen grain of an angiosperm was chosen for study because the daughter nuclei from this division have quite different fates.

2. MATERIALS AND METHODS

Species studied were *Zea mays* L. and *Agave attenuata* Salm-Dyck. Both species were diploid with regular meiosis and high pollen fertility. Buds were fixed in

10 per cent. neutral formalin. In agave, the perianth parts were removed and the tips of anthers were cut. A single anther from a bud was examined and, if pollen was found to be undergoing the first mitosis, the rest of the bud was retained for further study. Selected buds were washed overnight in running water, dehydrated in tertiary butyl alcohol and embedded in paraffin wax (Johanssen, 1940, p. 130). Sections were cut 30 μ thick, mounted and stained by the method of Alfert and Geschwind (1953) in 0·1 per cent. fast green FCF (George T. Gurr, London) at *p*H 8·0 after extracting nucleic acids in 5 per cent. trichloroacetic acid (TCA) at 90° C. for 15 minutes. Following differentiation of the staining in 95 per cent. alcohol, slides were dehydrated and mounted in neutral mounting medium.

In their description of the staining method, Alfert and Geschwind (1953) used onion root tips as a representative plant tissue. As a check on the staining methods, root tips of onion and maize were fixed and embedded at the same time as anthers; slides carrying sections 12 μ thick were treated simultaneously with the sections of anthers.

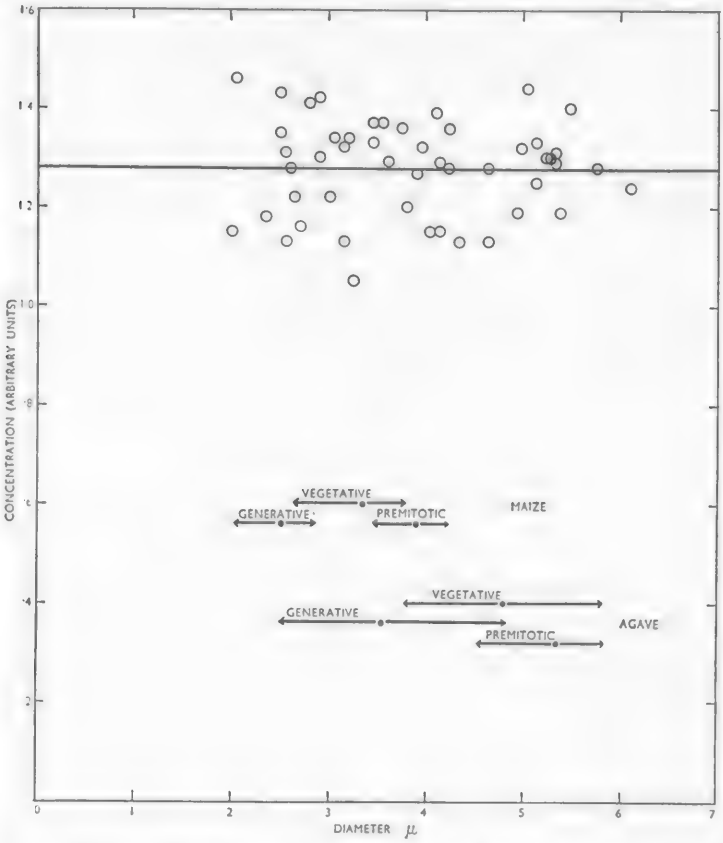
Unexpectedly, nucleoli in the pollen grains stained deeply. Between late prophase and telophase, the chromosomes also stained deeply. However, most pollen grains, including all those on which measurements were made, were either mononucleate and at late interphase or early prophase, or binucleate and at early interphase. In these, the nuclei were not suitable for microphotometric measurement because they were lightly stained and ill-defined in comparison with the nucleolus, to which all measurements were confined.

Slides of agave and maize were chosen for measurement if the sections of anthers contained mostly pre-mitotic pollen grains and relatively few post-mitotic. The positions of all post-mitotic pollen grains were noted and were then inspected in turn to see whether they could be measured. They were rejected only if (*a*) one or both nucleoli were at the upper or lower limit of the section and apparently cut, or (*b*) the two nucleoli were over-lapping or one or both screened by a fold in the pollen grain wall, debris or another pollen grain. Once selected, no pollen grain was subsequently rejected. Pre-mitotic pollen grains were numerous and the nearest to a measured post-mitotic grain was chosen for measurement unless rejected for one of the reasons already listed. It was thought that, in this way, a random sample was obtained. Thirty pre-mitotic and thirty pairs of post-mitotic nucleoli were measured on each slide. Two slides of maize and two of agave, each prepared at a different time, were used.

A screw micrometer eyepiece was used to measure the nucleolar diameters and the average of two measurements at right-angles was taken as the nucleolar diameter for calculating volume. Extinctions were measured with the Lison histophotometer (Electrophysique S.A., Brussels). A Philips E14 50-watt microscope lamp was used as a source of light. Using a 50× fluorite oil-immersion objective (N.A. 1·0) an image was projected on a screen in the centre of which was a hole of 2 mm. diameter. This was equivalent to 70 per cent. of the diameter of the smallest nucleolus measured and 27 per cent. of the largest. Behind the hole was a Wratten 72B filter and behind this a photoelectric cell. A blank reading was taken immediately adjacent to the nucleolus to be measured and the galvanometer adjusted to 100. The slide was then moved so that the nucleolus was centred over the hole and the transmittance read. The amount of fast green stain, in arbitrary units, was calculated from the formula $\frac{EC^2}{F}$ where E is the extinction $\left(\log_e \frac{1}{\text{Transmittance}}\right)$ and F the fraction of the volume of the nucleus included in the central plug, of radius c, whose extinction was measured. $F = \frac{1-(r^2-c^2)^{\frac{3}{2}}}{r^3}$ where r is the radius of the nucleus, assumed to be spherical (Swift, 1950).

An estimate of the accuracy of the method was obtained as follows. A solution of polyvinyl alcohol in water, and of approximately the same refractive index as

paraffin oil, was used to dissolve fast green FCF. A drop of this solution was then mixed with paraffin oil on a slide, covered with a cover slip, and the resulting spherical bubbles were examined. To prevent flattening and movement under the oil-immersion objective, the slide had several 20 μ de-waxed sections adhering to it. Fifty bubbles were measured under conditions similar to those used in the maize and agave experiments and over the same range of diameters. The fraction $\frac{\text{amount}}{\text{volume}}$, *i.e.* the concentration, which should have been constant, was plotted against the



TEXT-FIG. 1.—Above: Measurements of the concentration of stain in spherical aqueous droplets in paraffin oil show that the standard error inherent in the method is 8 per cent. Errors are distributed at random with respect to diameter of droplet. Below: The means and ranges of the diameters of the six classes of nucleoli studied.

diameter. The results are given in the upper part of fig. 1. The standard deviation was 8 per cent. of the mean but the errors were distributed at random to the size of the droplets. This variance, though larger than might have been desired, would add a random but not a systematic error to the measurements of the experiments.

3. RESULTS

In the onion root tips the staining was similar to that described by Alfert and Geschwind (1953); *i.e.* it was confined to the nuclei but not the nucleoli and, in mitotic cells, to the chromosomes (plate, fig. 1). In the pollen grains, the stain was also confined to nuclei and chromosomes but, as already mentioned, the nucleoli stained deeply and consistently (plate, figs. 2-7). That this was a tissue and

TABLE 1

Amounts of fast green (in arbitrary units) in nucleoli of pollen grains

Nucleus	Maize, slide 1	Maize, slide 2	Agave, slide 1	Agave, slide 2
Vegetative	1·48±0·33	1·36±0·26	3·99±1·03	3·62±0·61
Generative	0·78±0·18	0·71±0·19	1·61±0·58	1·47±0·28
Sum of vegetative+generative	2·26±0·45	2·07±0·40	5·60±1·48	5·09±0·81
Pre-mitotic	2·37±0·36	2·12±0·24	5·40±1·28	5·18±0·79

not a species difference was shown by staining maize root tips in which the nucleoli, as in onion, remained unstained.

The nucleolus was regularly circular in outline, *e.g.* in the vegetative nuclei of agave, the mean of the differences between the two measured

TABLE 2

Concentrations, in arbitrary units, in nucleoli

Slide	Nuclei	Concentration
Maize no. 1 . . .	Vegetative	1·60±0·30
	Generative	1·88±0·46
	Pre-mitotic	1·55±0·22
Maize no. 2 . . .	Vegetative	1·47±0·28
	Generative	1·80±0·41
	Pre-mitotic	1·41±0·23
Agave no. 1 . . .	Vegetative	1·37±0·20
	Generative	1·36±0·42
	Pre-mitotic	1·36±0·22
Agave no. 2 . . .	Vegetative	1·37±0·20
	Generative	1·31±0·28
	Pre-mitotic	1·40±0·05

diameters was 5·7 per cent. of the mean diameter. More than one nucleolus per nucleus was never observed. There was never any difficulty in telling the generative from the vegetative nucleus. They could be distinguished even at the earliest stage when they were still nearly touching.

Results are shown in table 1. The amount of stain in the vegetative nucleoli significantly exceeds that in the generative ones in all cases.

In maize, the amount in the vegetative nucleolus was approximately double that in the generative nucleolus and, in agave, more than double. In all four experiments the mean of the sums of the amounts of stain in the pairs of vegetative and generative nucleoli did not differ from the mean of the amount in the pre-mitotic nucleoli.

In table 2 the concentrations of stain (calculated from the amount of stain and volume) in the different classes of nucleoli are shown. In agave the concentrations in all three classes were the same and, in maize, the vegetative and pre-mitotic nucleoli did not differ. But the concentration in the generative nucleoli in maize was approximately 24 per cent. higher than in the pre-mitotic nucleoli and 20 per cent. higher than in the vegetative nucleoli, these differences being highly significant.

4. DISCUSSION

The observation that, under apparently similar conditions, the nucleoli in onion and maize root tips do not stain, whereas in the pollen grains of maize and agave they do, suggests that nucleoli may vary in their content of basic protein. There has been some confusion on this subject, Caspersson (1950) and Serra and Queirez Lopez (1944) having reported that nucleoli contain basic protein while Pollister and Ris (1947) and Vincent (1952) were unable to confirm this. The observations presented here give some direct evidence in support of the conclusion of Vincent (1955) that nucleoli might contain varying amounts of basic proteins depending on the metabolic activities of the cells.

On their own, the data in table 1 may be taken to mean that the stain was specific to basic protein, that the basic protein of the pre-mitotic nucleolus was divided unequally and without loss to the daughter nuclei where it was reconstituted into nucleoli which were measured before any new synthesis occurred after mitosis. The data in table 2 make it necessary to re-examine these conclusions. The observations that the concentration of stain was the same in all three classes of nuclei in agave and was also the same in two classes of nuclei in maize, permit of two alternative explanations. Either the stain was specific for basic protein and the amount of this determined the volume of the measured body; or the stain was not specific for basic protein but was taken up in the same concentrations by each nucleolus, the amount of stain merely reflecting its volume. It is not possible to choose rigorously between these two alternatives but the former seems more likely for two reasons. Firstly, the experiments of Alfert and Geschwind (1953), Ansley (1954), Bloch and Godman (1955*a*, 1955*b*), Alfert (1956) and Horn and Ward (1957) suggest that the stain is specific for basic proteins and the evidence already presented on localisation of the stain does not give any reason to doubt this specificity. Secondly, the aberrant class of nucleoli in table 2 (the generative nucleoli of maize) was the smallest of all six classes. If the stain had

been taken up in equal concentration in all nucleoli, this would imply that the concentration had been overestimated in the smallest nucleoli. This seems unlikely from the results shown in fig. 1. Moreover, as the diameter became smaller and approached the diameter of the plug, one would expect that consequent errors, arising from eccentricity, would have resulted in an underestimation of concentration. It seems more likely that, in the generative nucleolus of maize, the amount of stain correctly indicated the amount of basic protein but the relationship between amount of basic protein and the measured volume broke down for some unknown reason. The most reasonable interpretation of the data is that, at mitosis, the basic protein of the pre-mitotic nucleolus was divided unequally and without loss between the vegetative and generative nucleoli.

The idea of a self-duplicating capacity of the nucleolus is not new. Brachet (1957, p. 153) states, " This cell organelle is supposed to disappear at prophase and to be re-synthesised at telophase. But it is by no means impossible that essential constituents of the nucleoli are equally distributed into the two daughter cells by the chromosomes ". The observations that the sums of the amounts of fast green in the two post-mitotic nucleoli equalled that in the pre-mitotic nucleoli suggests that basic proteins are so distributed. In these experiments, however, the distribution was unequal.

The mechanism by which this might be effected is obscure. RNA (Swift *et al.*, 1956), zinc (Fujii, 1954) and alkaline phosphatase (Chevremont and Firket, 1953) appear on the chromosomes as soon as the nucleoli disappear. Vincent (1955) concluded that, at telophase, nucleolar materials are found along the surface of the entire chromosome complement and that these materials aggregate and fuse at the site of the nucleolar organiser. Observations in the present work are not incompatible with this scheme (plate, figs. 4-7). If it is correct, it suggests that, when the nucleolus disintegrates at prometaphase, basic proteins are distributed over the chromosome complement and, at the following telophase, stripped off to form new nucleoli. A consistently unequal distribution at anaphase could be caused by an uneven distribution of basic proteins at prometaphase and a response of all the more heavily charged daughter chromosomes to a gradient along the spindle. That such a gradient exists at the first pollen grain mitosis was shown by La Cour (1949) and Müntzing (1946).

Although the mechanism must remain speculative, the evidence does suggest that there is an unequal distribution of nuclear material at mitosis. This, like the uneven distribution of cytoplasmic material which precedes it (La Cour, 1949), may be important in the differentiation of the nuclei of pollen grains.

5. SUMMARY

1. Using a fast green staining procedure thought to be specific for basic proteins, it was found that nucleoli in pollen grains of *Zea mays*

Plate

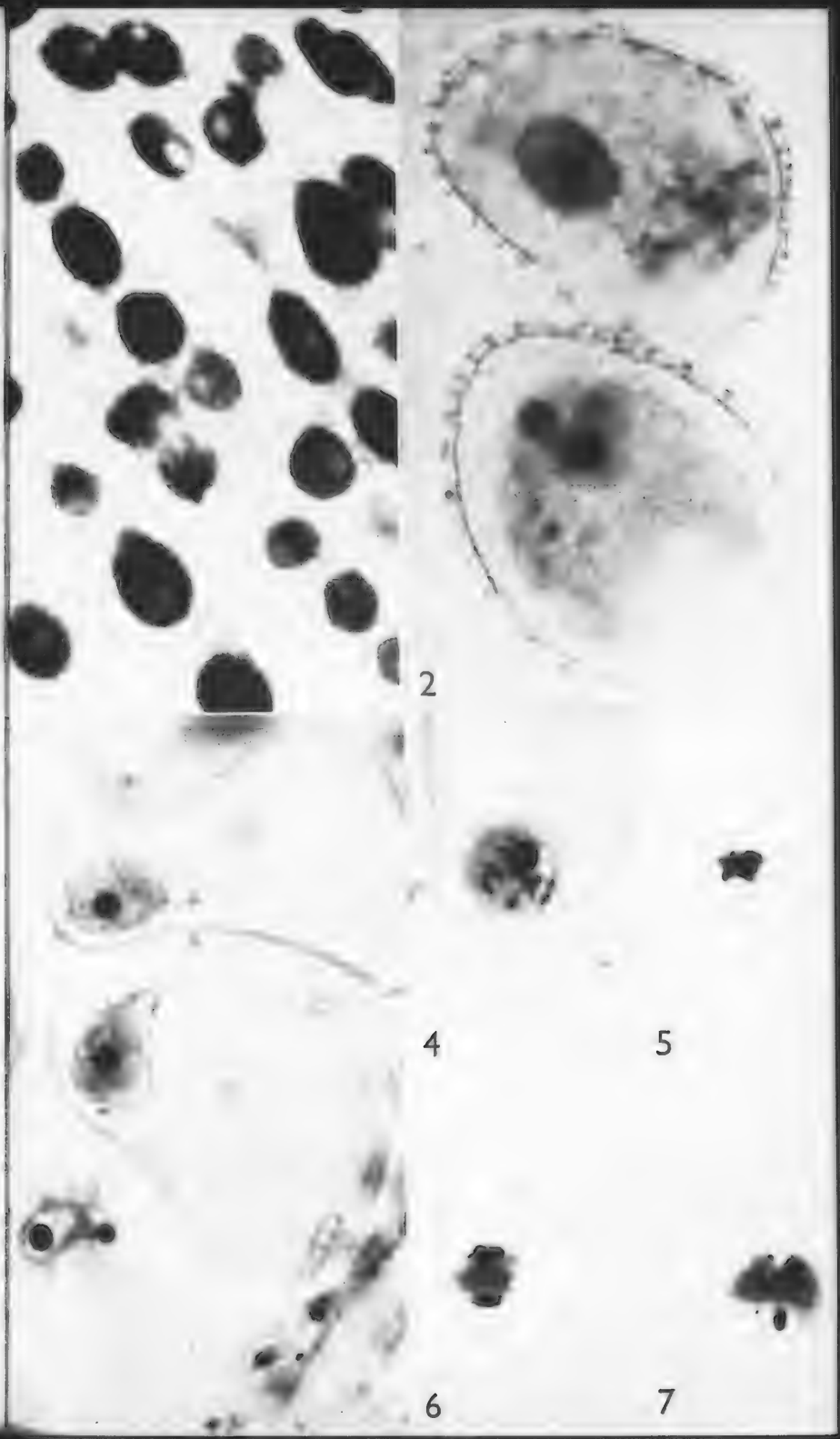
In each figure the stain used was fast green FCF at pH 8·0 following fixation in neutral 10 per cent. formalin and removal of nucleic acids. All magnifications ×940.

FIG. 1.—*Allium cepa*. Root tip showing nuclei and chromosomes stained, nucleoli not stained.

FIG. 2.—*Agave attenuata*. Pollen grains showing nucleoli typical of those on which measurements were made. Top, pre-mitotic ; bottom, post-mitotic.

FIG. 3.—*Zea mays*. Pollen grains showing nucleoli typical of those on which measurements were made. Top and centre, pre-mitotic ; bottom, post-mitotic.

FIGS. 4-7.—*Zea mays*. The first mitosis in the pollen grain ; 4—late prophase showing nucleolus and chromosomes ; 5—metaphase ; 6—telophase ; 7—late telophase, nucleoli reforming.


2
4
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6
7

and *Agave attenuata* stained, while nucleoli in the root-tips of *Zea mays* and *Allium cepa* did not stain.

2. Unequal amounts of fast green stain were present in the generative and vegetative nucleoli after the first mitosis in the pollen grains of *Zea mays* and *Agave attenuata*.

3. The sum of the amounts in the post-mitotic nucleoli did not differ from the amount in the pre-mitotic nucleoli.

4. The data are interpreted as meaning that, at mitosis, basic protein of the pre-mitotic nucleolus was divided unequally and without loss between the vegetative and generative nucleoli.

5. The possibility that this may be a cause of differentiation is discussed.

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6. REFERENCES

ALFERT, M. 1956. Chemical differentiation of nuclear proteins during spermatogenesis in the salmon. *J. Biophys. Biochem. Cytol.*, *2*, 109-114.

ALFERT, M., AND GESCHWIND, I. 1953. A selective staining method for the basic proteins of cell nuclei. *Proc. Natl. Acad. Sci. U.S.*, *39*, 991-999.

ANSLEY, H. R. 1954. A cytological and cytophotometric study of alternative pathways of meiosis in the house centipede (*Scutigera forceps* Rafinesque). *Chromosoma*, *6*, 656-695.

BLOCH, D. P., AND GODMAN, G. C. 1955*a*. A microphotometric study of the synthesis of DNA and nuclear histone. *J. Biophys. Biochem. Cytol.*, *1*, 17-28.

BLOCH, D. P., AND GODMAN, G. C. 1955*b*. Evidence of differences in the deoxyribonucleo-protein complex of rapidly proliferating and non-dividing cells. *J. Biophys. Biochem. Cytol.*, *1*, 531-550.

BRACHET, J. 1957. *Biochemical Cytology*. New York, Academic Press Inc.

CASPERSSON, T. O. 1950. *Cell Growth and Cell Function*. Norton, New York.

CHEVREMONT, M., AND FIRKET, H. 1953. Alkaline phosphatase of the nucleus. *Intern. Rev. Cytol.*, *2*, 261-288.

CRUFT, H. J., MAURITZEN, C. M., AND STEDMAN, E. 1954. Abnormal properties of histones from malignant cells. *Nature*, *174*, 580.

FUJII, T. 1954. Presence of zinc in nucleoli and its possible role in mitosis. *Nature*, *174*, 1108.

HORN, E. C., AND WARD, C. L. 1957. The localisation of basic proteins in the nuclei of larval Drosophila salivary glands. *Proc. Natl. Acad. Sci. U.S.*, *43*, 776-779.

JOHANSSEN, D. A. 1940. *Plant Microtechnique*. McGraw-Hill Book Co. Inc., New York and London.

KING, T. J., AND BRIGGS, R. 1955. Changes in the nuclei of differentiating gastrula cells, as demonstrated by nuclear transplantation. *Proc. Natl. Acad. Sci. U.S.*, *41*, 321-325.

LA COUR, L. F. 1949. Differentiation in the pollen grain. *Heredity*, *3*, 319-337.

MÜNTZING, A. 1946. Cytological studies of extra fragment chromosomes in rye. III. The mechanism of non-disjunction at the pollen mitosis. *Hereditas*, *32*, 127-129.

POLLISTER, A. W., AND RIS, H. 1947. Nucleoprotein determination in cytological preparations. *Cold Spring Harbor Symp. Quant. Biol.*, *12*, 147-157.

SERRA, J. A., AND QUEIREZ LOPEZ, A. 1944. Direkter Nachweis basischer Proteine in den Chromosomen und im Nukleolus. *Naturwissenschaften*, *32*, 47.

STEDMAN, E., AND STEDMAN, E. 1947. The chemical nature and functions of the components of cell nuclei. *Cold Spring Harbor Symp. Quant. Biol.*, *12*, 224-236.

SWIFT, H. H. 1950. The deoxyribose nucleic acid content of animal nuclei. *Physiologic. Zool.*, *23*, 169-198.

SWIFT, H. H., REBHUN, L., RASCH, E., AND WOODARD, J. 1956. The cytology of nuclear RNA. In *Cellular Mechanisms of Differentiation and Growth* (D. Rudnick, ed.), pp. 45-59, Princeton Univ. Press, Princeton, New Jersey.

VINCENT, W. S. 1952. The isolation and chemical properties of the nucleoli of starfish oocytes. *Proc. Natl. Acad. Sci. U.S.*, *38*, 139-145.

VINCENT, W. S. 1955. Structure and chemistry of nucleoli. *Intern. Rev. Cytol.*, *4*, 269-298.

HETEROSIS AND THE INHERITANCE OF YIELD IN THE TOMATO

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1. INTRODUCTION

THE diallel cross has achieved considerable popularity in recent years. Our excuse for reporting another one is that ours is much bigger than the others. It provides a worthwhile number of degrees of freedom for comparisons between parents, and so permits an attempt at formulating rules for the breeding of self-fertilising plants such as the tomato. General rules of plant breeding procedure are still conspicuously lacking, and genetical theory contributes less than is generally admitted to crop improvement except in the simpler kinds of character transference. The complexity of the inheritance of quantitative characters defies even the most sophisticated theoretical attempts at a measure of rationalisation that would be helpful in the prediction and manipulation of progenies. It has been decided therefore in describing the present material that consideration other than of the phenotypic behaviour of the parents and crosses would be of little use.

One of the chief interests in general breeding work in recent years has been in the utilisation of heterosis. The most striking success in this field has been in out-breeding species, particularly maize in which separation of the sexes leads to economical production of hybrid seed. With inbreeding crops such as the tomato, the practical importance of heterosis is less clear (Williams, 1959*a*), and it is doubtful whether hybridity *per se* has any advantage for increasing character expression when compared with pure lines. The replication afforded by the present diallel cross can assist in determining the relative importance of heterosis in self-fertilising crops.

Gilbert (1958) following an analysis of all available evidence from diallel crosses in several different species concluded that the value of the diallel cross in plant breeding should not be exaggerated, and that the information gained from it is very little more than that obtained from the parents themselves. In this work it is proposed to expose some of the details underlying this generalisation and by so doing to draw attention to certain aspects of behaviour of the parent varieties which are reproducible over a wide range of crosses.

2. MATERIALS AND METHODS

The parents were Vetomold, Kondine Red, Stonor's Exhibition, Ailsa Craig, Harbinger, Potential, Potentate, Vagabond, Leafmould Resister 1, Plumpton King,

Radio, Moneymaker, Comet, Crackerjack, Baby Lea, Downes' Seedling, E.S.1 and Delicious. Most of these varieties have been under observation here for many years, and as far as can be determined the samples were true to name. The F_1's and parents were grown in a glasshouse in 1957, using a lattice square design with six replicates and one plant per plot. In 1958 the F_1's and parents were grown out of doors in a lattice square with two replicates and three plants per plot.

The yields (in ounces) and fruit numbers are given on a per-plant basis, so that the F_1 figures (and the parents grown with them) for each year are the means of six plants each. The average fruit weights (ounces) were calculated from the total yields of three plants each, so that the figures quoted here are the means of two average fruit weights per cross per year. All figures presented refer to records taken during the first three weeks of fruiting.

3. THE PERFORMANCE OF THE PARENTS

Apart from the few varieties released following systematic breeding by Stonor (unpublished), Bewley (1930-53) and Crane (1951), the majority of tomato varieties at present in cultivation in England are of uncertain origin. Of the 18 varieties used in the present diallel cross only the origins of Vagabond, Vetomold, L.M.R. No. 1 and E.S.1 are known with certainty. Exhibition and Moneymaker are known to have been derived from the " green-top " (*U*) form by introducing the gene *u* for uniform fruit colour from Devon Surprise, but no information is available on the other parents used in the crosses. The range of genetic variation in English tomato varieties cannot therefore be assessed and one is probably dealing in many cases with very similar types and perhaps even with synonyms. The work described here can assist in the resolution of genetic differences between the varieties used because their contributions to 17 different hybrids in two different environments have been determined. This diallel cross provides extensive data on the transmission of characters by the parents, and enables one to assess the extent of genetic diversity in agronomically similar varieties.

Some of the 18 varieties fall into two fairly well defined groups.

Groups A and B differ in average fruit weight and in fruit number. Varieties in group A produce a large number of small fruit, giving a large yield, while those in group B have a smaller number of large fruit and a poor yield. These groups are characterised not merely by these numerical observations but also by the strikingly similar appearance of varieties in each group.

It will be noted that none of the 18 varieties combined maximal fruit number with maximal fruit weight. At their upper limits, these two components of yield are almost certainly mutually exclusive. The correlation between average fruit weight and fruit number in these 18 parents was −0·66.

The most important component of yield, given the range of average fruit weights in the present series of varieties, is fruit number. The correlation between fruit number and early yield is 0·80. Since varieties with respectively smaller or larger fruits than the smallest

or largest represented here have little use commercially, it can be concluded that in the cultivated tomato maximal yield can only be achieved in forms which have the capacity to ripen large numbers of fruit.

Earliness of ripening in the tomato is governed by rate of development during two stages of growth of the seedling (Williams, 1959*b*).

TABLE 1

The levels of yield and yield components in 18 parent varieties. First 3 weeks' yield 1957+1958

Variety	Yield (±1·75)	Fruit no. (±0·64)	Average fruit weight (±0·177)
Group A			
Ailsa Craig	16·8	9·2	1·85
Harbinger	16·6	7·1	2·18
Plumpton King	14·4	7·0	2·08
Crackerjack	9·8	5·2	1·92
Group B			
Vetomold	11·2	3·9	2·96
Potentate	11·0	3·7	2·86
Potential	9·3	3·2	2·99
Vagabond	6·8	2·2	3·13
Comet	11·0	3·3	3·36
Baby Lea	10·4	3·3	3·29
Other varieties			
Kondine Red	8·5	3·6	2·25
LMR 1	12·0	5·0	2·74
E.S. 1	9·0	4·7	2·12
Delicious	6·4	3·1	2·12
Radio	6·6	3·2	2·39
Downes' Seedling	6·4	3·5	1·98
Exhibition	2·8	1·0	2·32
Moneymaker	6·9	2·8	2·99

In different varieties the rate of development to first flower relative to that from first flower to first ripe fruit frequently differs. In the present series of parents the time to flowering under glass in varieties of group A was on average greater than in those of group B. Since group A varieties produced a significantly greater number of early fruit than those in group B it is clear that they develop at a far more rapid rate than the latter during the period from flowering to fruit ripening. Hybrid combinations between varieties which develop

rapidly during different periods gave the best early yield in the F_1 families.

Table 2 compares the behaviour of parent varieties in the two years.

In this and in some later tables the best and poorest parents have been selected on their performance in 1957. Table 1 shows that different sets of five must be selected for different characters. It must be remembered that a " poor " variety might be selected, not because it is an inferior performer in that environment, but because the six plants representing it happened to be rather poor specimens. Such a variety could be expected to recover even if grown again in identical conditions. The act of selection may bias our figures. The fact remains that the parents behave differently (*cf.* Appendix) and that this

TABLE 2

Average performance of parents in 1957 and 1958

Character	General average	Average top 5, 1957	Average bottom 5, 1957
Yield 1957	8·8	13·6	3·9
1958	10·8	11·9	8·5
Fruit number 1957	3·2	5·7	1·4
1958	5·1	7·6	4·1
Average fruit weight 1957	2·86	3·63	2·13
1958	2·20	2·61	1·91

has to be considered in discussion of the F_1 results. The five best varieties under glass showed a drop in early yield when grown outdoors, while the poorest increased by 120 per cent. Similarly with regard to fruit number, the best varieties improved only 33 per cent. when field grown while the varieties with low fruit number showed an increase of nearly 200 per cent. The most important difference, however, was in average fruit weight. The difference in general average fruit weight between the two years is apparently due to a reduction mainly in varieties with large fruit. It is worth noting that between 1957 and 1958, fruit number increased and fruit size decreased in these varieties, and that the fluctuations compensated differently in the different varieties, thus producing relative differences in yield. As will be shown later, this unequal behaviour of the varieties can affect any conclusions regarding heterosis in the F_1 families.

4. THE PERFORMANCE OF F_1 HYBRIDS

It has been shown (Gilbert, 1958) that the average performance of hybrids is closely related to the behaviour of the parents. The best parent for a particular character gave on average the best set of F_1 families in the diallel cross. Interactions between parents (*i.e.* specific

combining abilities) were negligible and the interactions that did occur varied widely from year to year (see Appendix). Thus it appears that the principle of knowing the best and breeding from them cannot be improved in any radical way. However, in order to allow this generalisation to be used in the most purposeful way in a breeding programme it is necessary to examine the data in somewhat greater detail.

Williams (1959*c*) suggested that heterosis for yield was the consequence of multiplicative relationships on the phenotypic or somatic level (Mather, 1949) between the component characters of the yield complex. Yield in the tomato is primarily made up of fruit number and average fruit weight, and heterosis for total early yield (weight), can occur in hybrids in which the component characters merely show dominant or intermediate levels of expression. For this to occur the parents must differ as regards the level of expression of each of the components and neither must have a monopoly of high (or low) expression in both the unit characters. Thus yield heterosis becomes simply an expression of an inequality known to mathematicians as Tchebychev's. The following is a simple case:

	1st component of yield	2nd component of yield	Yield
Parent A . .	1	3	3
Parent B . .	3	1	3
F_1 (A×B) . .	2	2	4

These preliminary conclusions can now be supported by the far more extensive data from the 18×18 diallel cross.

The " top-parent " in each cross is the one that gives the best performance. Where the F_1 mean in table 3 is no greater than the " top-parent " mean, the F_1 families *on average* do not exhibit heterosis. Apart from average fruit weight in 1958, which will be discussed in greater detail later, the hybrids show no heterosis for the components of yield. Yield itself, on the other hand, is clearly heterotic in the two environments.

In this connection reference may be made to the 10×10 diallel cross between tomato lines reported by Powers (1945). Although the enormous phenotypic differences between the parents used make the averages rather unsatisfactory, they can be used for present purposes. A summary of Powers' data similar to that given in table 3 is given in table 4.

The standard errors of different varieties were heterogeneous and this has been allowed for in calculating the S.E. of the average " top-parent ". Examination of individual crosses reveals heterosis for yield. Fruit weight shows no heterosis and fruit number is heterotic only in the three crosses between the three lowest parents.

Thus in all these data, heterosis for yield is a property of the phenotype requiring no special genetical explanation. It must be understood

TABLE 3

Mean values of all F_1 families

		Average yield	Average fruit no.	Average fruit weight
1957	F_1	13·0 (±0·17)	4·7 (±0·06)	2·92 (±0·023)
	Top-parent *	11·1 (±0·58)	4·3 (±0·21)	3·24 (±0·079)
1958	F_1	15·4 (±0·23)	6·0 (±0·08)	2·62 (±0·017)
	Top-parent	13·2 (±0·78)	6·4 (±0·28)	2·45 (±0·058)

* The error variance of the mean of the "top-parent" in a $k \times k$ diallel cross is $\frac{2(2k-1)}{3k(k-1)}\sigma^2$, where σ^2 is the error variance of one parent.

that the complexity of the component characters is also very great and that consequently we are no nearer to consideration of basic cell

TABLE 4

Mean values of all F_1 families from Powers (1945)

	Yield gm. per plant	Fruit number per plant	Average fruit weight (gm.)
F_1	1437±20·7	35·9±0·59	66±0·6
"Top-parent"	1152±52·4	51·1±3·3	102±3·3

processes. Fortunately this need not concern us here since our primary interest is in the phenotype.

5. THE RELATIONSHIP BETWEEN PARENTS AND F_1 HYBRIDS

In the absence of sizable interaction, the main effects derived from arrays of 17 hybrids involving a common parent in the diallel are a measure of which parents yield the best hybrids.

Table 5 shows that the main effects correspond to the parental order with respect to all the characters although differences do exist which are statistically—and, we must presume, genetically—significant (see Appendix). In fact, as with the parents, the best hybrid series are those which produce high fruit numbers at the expense of fruit size. An examination of the individual hybrids in the arrays shows that the majority of the high yielding hybrids are to be found in crosses involving Ailsa Craig and Harbinger. In relation to general economy in a breeding programme, extensive "top-crosses" with

either or perhaps both of these parents would have resulted in the identification of the greater proportion of the most promising crosses.

The equality of these two varieties with respect to yield characters, and the high degree of correspondence between their F_1 hybrids raise doubts as to whether they are in fact distinct varieties, and highlights

TABLE 5

Correlations between main effects and parental values
(Each correlation has 16 d.f.)

	Yield	Fruit no.	Fruit weight
1957	0·62	0·78	0·82
1958	0·58	0·73	0·80

the question of synonyms mentioned earlier. Their breeding behaviour suggests that, at least for all practical purposes, they can be considered to be identical.

The performance of the hybrids is given in table 6.

TABLE 6

Mean value of hybrids in 1957 and 1958

Character and year	General average (153 hybrids)	Average top five arrays (1957) *	Average bottom five arrays (1957)*
Yield (oz.) 1957	13·0	15·7	10·6
1958	15·4	17·6	13·9
Fruit number 1957	4·7	6·1	3·7
1958	6·0	7·0	5·4
Av. fruit weight (oz.) 1957	2·92	3·26	2·54
1958	2·62	2·76	2·38

* The top and bottom five arrays were selected on the 1957 data. The arrays taken need not be the same for each of the three characters. Those hybrids which occur in two of the five arrays are counted twice.

The tendency towards equalisation in yield in 1958 which occurred in the parents does not occur in the F_1 hybrids (*cf.* Appendix). The hybrids gave a more consistent performance under the highly contrasting conditions of the two environments both as regards yield and the component characters of yield.

6. HETEROSIS FOR YIELD AND COMPONENT CHARACTERS OF YIELD IN INDIVIDUAL CROSSES

The prevalence of heterosis in any crop species has practical as well as theoretical implication since success in the production of superior-hybrids will depend greatly on the frequency of heterotic combinations. An assessment of the occurrence of heterosis as a general

phenomenon as opposed to isolated cases is therefore considered to be of the utmost importance. It is impracticable in an extensive diallel cross such as the present one to undertake the extensive testing necessary to establish beyond doubt the presence of heterosis in each cross that shows excess over the top-parent. We consider therefore that the examination of groups of means within a large diallel cross is the only practical method of detecting heterosis as a general phenomenon.

It is frequently assumed in discussions on the practical utilisation of heterosis, that any F_1 hybrid which is recorded as being significantly

TABLE 7

Two 5×5 diallel crosses showing deviation of each hybrid from its top-parent (1957 yield)

(*a*) Five parents with high yield

	Plumpton King	Harbinger	Potentate	Vetomold
	($\pm$2·94)			
Harbinger	0			
Potentate	−7·7	+5·8		
Vetomold	−1·0	−4·2	−3·2	
Baby Lea	+0·8	+6·0	0	+1·7

(*b*) Five parents with low yield

	Exhibition	Delicious	Downes' Seedling	Moneymaker
	($\pm$2·94)			
Delicious	+6·0			
Downes' Seedling	+3·2	+6·3		
Moneymaker	+2·5	+1·2	+6·0	
Kondine Red	+13·2	+7·8	+9·8	+7·8

greater than the best parent in the given cross is support for the use of F_1 hybrids in practice. While heterosis might be recognised in crosses between parents of less than maximal expression, unless it achieves a level greater than in the best parents available, it can have no practical significance. To establish the existence of heterosis for yield in any given cross may be of some theoretical interest, but unless it can be shown that the level achieved is equal to or better than that of the best parent available, the exercise is futile in terms of better tomato varieties.

The mean yield of the F_1 families produced by the three best varieties was slightly less than would be expected on the assumption

of dominance of high yield. In other words the best varieties were in general better than their hybrids. Superiority of the hybrids over the best parent occurred in this material only when the parents used were below maximal levels. Thus it appears that the expression of heterosis in the tomato largely depends on the choice of parents. Table 7 illustrates this point.

If individual hybrids in table 7*a* are considered, it might be concluded that the combinations Potentate × Harbinger and Baby Lea × Harbinger are heterotic but this can only be definitely established

TABLE 8

Two 5 × 5 diallel crosses showing deviation of each hybrid from its top-parent (1957 fruit number)

(*a*) Five parents with high fruit number

	Plumpton King	Harbinger	Ailsa Craig	Crackerjack
	(±1·08)			
Harbinger	+0·7			
Ailsa Craig	−0·5	+4·5		
Crackerjack	−2·2	−0·7	+3·0	
E.S.1	−2·0	+1·0	+2·7	−1·0

(*b*) Five parents with low fruit number

	Exhibition	Moneymaker	Downes' Seedling	Delicious
	(±1·08)			
Moneymaker	+0·7			
Downes' Seedling	+0·7	+2·0		
Delicious	+1·3	+0·7	+3·0	
Comet	+2·5	+1·2	+2·7	+0·7

after further extensive testing. These two deviations are not significantly different from zero, whereas all ten values in 7*b* are positive and several are " significant ".

(i) *Fruit number*

The absence of heterosis in yield characters noted in a previous section referred to the overall behaviour of all the crosses. However, examination of the individual crosses shows that heterosis for fruit number is not absent in all the hybrids, and that (as in Powers' data) parents having low fruit numbers, in general give rise to hybrids in which number of fruit is heterotic (table 8). Varieties with high

fruit number are on average superior to their hybrids. Because of this, the average fruit numbers for top-parents and hybrids in the whole diallel are still equal (table 3). It appears that heterosis is completely absent in unitary yield characters only when one is dealing with genotypes capable of maximal expression in the character concerned. It must be pointed out however that table 8*a* involves all four members of group A, and as has already been noted, some of the varieties within this group are genetically very similar. Again, the conclusion that the positive deviations in table 8*a* are merely due to chance may be over-pessimistic, but is unavoidable without further data.

(ii) *Average fruit weight*

Under glasshouse conditions in 1957, the fruit weight of the F_1 hybrids agreed with the mid-parent value, while in the field trial the hybrids were on average heterotic for this character. It will be seen from tables 2 and 6 that the five large fruited parents were relatively much less stable as regards fruit weight than the large fruited hybrids under the two environments. Of the large fruited parents only Comet and Baby Lea gave similar fruit weights in the two tests, and these two varieties showed no average heterosis in their respective sets of hybrids in the 1958 test. The remaining large fruited varieties produced significantly lighter fruit and were inferior to their F_1 hybrids when grown in the field. The sudden appearance of heterosis for fruit weight in one season can therefore be explained by the behaviour of the parents. It is clear that the hybrids provide a better buffer against environmental influences than the pure lines, and that their relative stability is the property of their genotypes. The genetic system operating in both environments is, however, the same and the danger of ascribing speculative properties, such as over-dominance, to the genotype in individual experiments where heterosis is apparent could not be clearer.

(iii) *Flowering date*

Since the time taken from germination to flowering is one of the important factors determining earliness, a record is necessary of the observations on flowering date made on the present material. The mean flowering date of the 153 hybrids equalled the mean of the earliest parent. The mean deviation from expectation assuming phenotypic dominance of earliness was $-0{\cdot}32$ days ($\pm 0{\cdot}214$). The mean deviation in F_1 series each containing 17 hybrids from the five earliest parents was $+0{\cdot}69$ days while the comparable figure from the five latest parents was $-0{\cdot}70$ days. The mean flowering date of hybrids derived from early varieties was slightly later than the mean of the earliest parent, while hybrids from late parents were heterotic for earlier flowering. In this character also it appears that such heterosis as exists is a property of hybrids involving mediocre parents.

7. DISCUSSION

The results outlined here show certain simple patterns of behaviour in the breeding performance of cultivated varieties of the tomato. In all the characters studied, with the exception of average fruit weight when the material was grown under glass, the mean of the 153 hybrids equalled or exceeded the mean of the top-parent. In their distribution about this average, however, they followed the mid-parent (Appendix). The top-parent for flowering date is here regarded as the earliest parent. The following summary of the average of 153 deviations for each character will serve to emphasise the constancy of behaviour in the hybrids:

Character	Average deviation from :	
	Top-parent	Mid-parent
Yield	+1·8 (±0·60)	+4·2 (±0·52)
Fruit number . .	−0·4 (±0·22)	+1·4 (±0·19)
Average fruit weight .	−0·32 (±0·082)	+0·06 (±0·071)
Flowering date (days) .	−0·32 (±0·214)	−1·32 (±0·186)

The above figures refer to 1957 and, apart from the behaviour of fruit weight already noted, are similar to the 1958 records. The means for all crosses show heterosis only with respect to early yield. Since the general mean for fruit weight was intermediate between the mean of the parents, and since the general mean for fruit number showed no better than the top-parent, it seems clear that the greater part of the heterosis for yield observed in this experiment was of a kind resulting from alternation of high and low expression in the yield components in the parents. It appears therefore that it is quite misleading to talk in terms of heterotic yield genes unless one knows precisely whether the component characters show heterosis. The observation made here is that heterotic yield levels may be achieved in hybrids which, with respect to the component units, show no heterosis and that therefore there is no need to postulate a special genetic system to explain heterosis. This also was the view of Hagberg (1952), who concluded that most of the heterosis in *Galeopsis* material was of a type which he termed " combination heterosis ".

One feature of great importance that is consistent throughout these data, is the general absence of heterosis with respect to all characters where parents with a high level of expression are involved. Heterosis in the tomato is expressed most frequently in hybrids between the poorer genotypes. Inbreeding species which do not show losses in vigour on inbreeding can be expected to have achieved as a result of selection, maximal levels of character expression in pure breeding, relatively homozygous lines. This has been confirmed in the tomato

(Williams, 1959*a*). With reference to total early yield, and omitting consideration of subjective characters such as fruit quality, very few of the hybrids which showed heterosis, surpassed either the best non-heterotic hybrid or therefore the best parent. Thus although heterotic hybrids do occur, any value that the majority of them have in practice will depend on improvements in characters other than total early yield.

The occurrence of heterosis only among the hybrids of parents in the lower part of the range of variation indicates that in the tomato, and possibly in other inbreeders also, a near maximal level of expression in absolute yield has been achieved by selection. This does not presuppose that at some future date, following the introduction of new material, the present maximum cannot be exceeded. Furthermore, from the genetical standpoint, it appears that the best parents possess genotypes which are balanced at a very high level, while the poorer parents complement each other in a manner that leads to heterosis. Indeed, the entire series studied here seems to behave according to a simple scheme based on complementary factors with varying degrees of dominance. It must be emphasised that no precise meaning can be attached to the use of the term " complementary factor " in this connection, since the relative importance of inter-allelic interaction (Mather, 1949; Jinks, 1955) as opposed to somatic interaction (see p. 137) in determining heterosis in the material cannot be decided. The present results agree fully with the interpretation of heterosis given by Keeble and Pellew (1910). This somewhat surprising conclusion suggests that much of the obscurity that at present surrounds the problem of heterosis arises from the failure to recognise the component characters of complex phenotypic expressions.

8. SUMMARY

1. The behaviour of 153 hybrids derived from 18 parent varieties of tomato has been recorded in two different environments. The characters observed included yield, fruit number, fruit weight, and flowering date.

2. It is concluded that the greater part of the average heterosis for yield in the material is due simply to the relative distribution of the levels of the components of yield in the parents of a given cross. Instances are described of yield heterosis in crosses where the components of yield were not heterotic. In naturally inbreeding species, many other characters which exhibit heterosis probably behave in this manner. It is suggested therefore that it is erroneous to speak of heterotic genes for complex characters such as crop yield.

3. Heterosis in this material was largely confined to crosses between parents which were below observed maxima in the range of variation of a particular character. Near maximal levels have been fixed in the pure breeding varieties which fall in the upper ranges of variation,

and these are not exceeded by heterotic hybrids between poorer parents.

4. The behaviour of the entire range of hybrids is consistent with a controlling genetic system that is based on complementary gene action with an unknown, and possibly variable, degree of dominance.

5. In the Appendix it is shown that statistical interactions between parents fluctuate from generation to generation; that the discrepancy between the yield of a parent and its general combining ability cannot yet be ignored; and that parents transmit variability of performance, as well as average performance, to their offspring.

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9. REFERENCES

BEWLEY, W. F. 1930-53. Reports on tomato investigations. *Ann. Reps. Cheshunt Expt. St.*

CRANE, M. B. 1951. Tomatoes : hybrid vigour. *John Innes Ann. Rep.*, pp. 11-12.

GILBERT, N. E. 1958. Diallel cross in plant breeding. *Heredity, 12*, 477-492.

GRIFFING, BRUCE. 1950. Analysis of quantitative gene action by constant-parent regression and related techniques. *Genetics, 35*, 303-321.

HAGBERG, A. 1952. Heterosis in F_1 combinations in *Galeopsis*. *Hereditas, 38*, 221-245.

HALDANE, J. B. S. 1957. Karl Pearson. *Biometrika, 44*, 303-313.

HULL, F. H. 1946. *Maize Genetics Co-operation News Letter, 20*.

JINKS, J. L. 1955. Heterosis in diallel crosses. *Heredity, 9*, 223-238.

KEEBLE, F., AND PELLEW, C. 1910. The mode of inheritance of stature and time of flowering in peas *P. sativum*. *J. Genet., 1*, 47-56.

MATHER, K. 1949. *Biometrical Analysis*. Methuen & Co., London.

POWERS, L. 1945. Relative yields of inbred lines and F_1 hybrids of tomato. *Bot. Gaz., 106*, 247-268.

WILLIAMS, W. 1959*a*. The isolation of pure lines from F_1 hybrids of tomato and the problem of heterosis in inbreeding crop species. *J. Agric. Sci. 53*, 347-353.

WILLIAMS, W. 1959*b*. Investigations on the tomato : the control of early fruiting. *John Innes Ann. Rep.*, p. 13.

WILLIAMS, W. 1959*c*. Heterosis and the genetics of complex characters. *Nature, 184*, 527-530.

[APPENDIX

APPENDIX

PREDICTING PERFORMANCE IN F_1 AND F_2 GENERATIONS

NEIL GILBERT

1. This appendix considers the performance of F_1 families in the 18×18 diallel cross and of their F_2 progenies. These F_2 families were grown in 1958 in a lattice square with two replicates and ten plants per plot. Parents were included in the experiment; thus they occurred twice in the same field, once with the F_1's (six plants each) and once with the F_2's (20 plants each). The parents × experiments interaction did not signify, yet there was a considerable difference in average performance per plant. It is therefore not always permissible to lump these two sets of parents together.

2. *Constancy of F_1 families between years.* In 1957 the F_1 were grown in a glasshouse; in 1958 out of doors in unusually bad conditions. The analyses of variance are:

	Degrees of freedom	Mean Square for		
		Yield	Fruit number	Average fruit weight
Average main effects	17	764	203·9	4·636
Main effects × years	17	71	15·3	0·638
Average interactions	135	77	11·8	0·225
Interactions × years	135	68	11·5	0·156
Average parents	17	161	46·5	1·022
Parents × years	17	75	16·2	0·218
Error		37	4·9	0·126

The main effects show a very satisfactory stability. Their interactions with years are of the same order of magnitude as their interactions with each other.

3. *Top-parent regression.* It is clear from table 3 that the mean values show general phenotypic dominance of top-parent. A multiple regression on top- and bottom-parents may, therefore, give better prediction than simple regression on mid-parents. It should be remembered that we are no longer dealing with general mean differences but with deviations from the general means. The results are contradictory. In every case except 1958 F_1 yield and fruit number, the regression on mid-parent (with one degree of freedom) accounts for all but an insignificant part of the multiple-regression sum of squares and a regression on top-parent alone gives relatively poor prediction. In the two cases mentioned, however, the situation is reversed and the top-parent on its own gives the best prediction although the mid-parent is still a good predictor (*i.e.* the M.S. for regression on mid-parent is not far short of that for regression on top-parent). Consequently it is best to use mid-parents (and, therefore, main effects) in predicting the relative merits of crosses. The values of these mid-parent regression coefficients will be discussed below.

4. *Main effects.* The main effect, or general combining ability, measures the average performance of a variety as a parent. Of course, what the breeder really wants to know is the contribution that a variety makes to the best of, say, its F_4 descendants. From the algebraic identities given by Gilbert (1958), p. 483, it will be seen that the regression coefficient of main effect on parental yield equals

one-half that of the yield of each cross on its mid-parent, whatever the actual values of these regressions. Since a cross gets half its genotype from each parent the regression of main effect on parental value might be expected to equal one-half, apart from some environmental dilution. In the previous paper I stated that this regression "does not, on average, differ from half". The present data contradict this.

	Regressions of main effects on parental values		
	Yield	Fruit number	Average fruit weight
1957 F_1	0·336 (±0·0297)	0·500 (±0·0243)	0·421 (±0·0263)
1958 F_1	0·231 (±0·0370)	0·246 (±0·0237)	0·416 (±0·0294)
1958 F_2	0·302 (±0·0226)	0·280 (±0·0106)	0·557 (±0·0403)

Now the above regression coefficients will be less than their "genetic" values owing to dilution by environmental variation. "Genetic" regressions were obtained by correcting for this (assuming that the "genetic" and "error" components of variance are additive), just to see what happened. It must be pointed out, however, that the error variance is shown below to change from variety to variety. The regressions still did not come up to expectation. When comparing main effects with parental values, therefore, it is necessary to abandon the "heterogeneity of potence" method which assumes a regression coefficient of one-half, and substitute a regression analysis. The main effects and parents are then compared in a relative, rather than an absolute, way. It will be seen below that the "heterogeneity of potence" idea is still of some value in another connection.

The situation can be summed up as follows; in the whole diallel cross, the mean of the hybrids usually exceeds the parental mean. In their deviations from their mean, the individual hybrids follow the corresponding mid-parents; but these deviations of the hybrids, although closely correlated with the deviations of the mid-parents from the parental mean, are not so large. The hybrids are distributed relatively compactly in the upper part of the parental range. Thus although the poorer parents show heterosis, the best parents do not.

In 1958 the F_2, F_1 and parents were all grown in the same field. Appealing to the fact that, on average, the amount of heterozygosity in the F_2 is half that in the F_1, it might be expected that F_2 main effect $= \frac{1}{2}$ F_1 main effect $+ \frac{1}{2}$ (parental value). The multiple regression of F_2 main effect on F_1 main effect and parent gives:

Yield	$0{\cdot}665t+0{\cdot}666p/2$
Fruit number . . .	$0{\cdot}312t+0{\cdot}287p/2$
Average fruit weight . .	$0{\cdot}523t+0{\cdot}663p/2$

Here t and p are themselves highly correlated, and reduction of their variances by subtracting the "error" component makes nonsense of these multiple regressions; in other words, the attempt to calculate a "genetical" multiple regression is a failure. The multiple regressions quoted show that, as before, the coefficients may differ from half. However, $t+p/2$ gives good relative prediction of the F_2 main effect.

None of these regressions show any sign of curvature. Altogether, the main effects behave satisfactorily, although not quite so well as might be hoped.

5. *Interactions.* The interaction, or specific combining ability for a particular cross, is the remainder when the general mean m and the two relevant main effects t have been subtracted from the F_1 yield. Since real interactions do occur, it is

interesting to see how constant they are from year to year and generation to generation. The correlations observed (each with 134 d.f.) are:

	Yield	Fruit number	Average fruit weight
1957 $F_1 \times$ 1958 F_1 .	0·069	0·017	0·179
1957 $F_1 \times$ 1958 F_2 .	0·111	0·106	0·218
1958 $F_1 \times$ 1958 F_2 .	0·152	0·062	0·019

Subtraction of the relevant error components does not increase the correlations to the region of 0·6 which may be considered the minimum value necessary for prediction in plant breeding. Furthermore, in practice error is always with us and is unlikely to be reduced sufficiently for the "genotypic" component of interaction to be estimated at all precisely. Interactions apparently vary so much from year to year and generation to generation that they can be forgotten when deciding on the best parents.

Since, on average, the genotype of a cross is composed of half the genotypes of each parent, we can tentatively call the difference between parental yield p and $m+2t$ (m = general parental mean, t = main effect for that parent) an interaction. From Gilbert (1958) it appears that these differences, labelled "heterogeneity of potence", are no more variable than the interactions. If this were not the case, we should have to ascribe some extraordinary property to the very condition of inbreeding, quite apart from the difference between the parental and F_1 averages. These "heterogeneities of potence" are more accurately estimated than the ordinary interactions. They give the following correlations (each with 16 d.f.):

	Yield	Fruit number	Average fruit weight
1957 $F_1 \times$ 1958 F_1 .	−0·030	−0·184	0·391
1957 $F_1 \times$ 1958 F_2 .	−0·198	−0·475	0·365
1958 $F_1 \times$ 1958 F_2 .	0·454	0·613	0·258

Bearing in mind the differences in the experimental conditions in 1957 and 1958 and also that main effects (covering all crosses involving one parent) are intrinsically more interesting than interactions (involving one cross only) it appears that for purposes of prediction, interactions are best forgotten but that "heterogeneities of potence" cannot yet be so dismissed.

In connection with interactions the technique of constant-parent regression introduced by Hull (1946) might be mentioned. For each parent, the regression coefficient of the yields of the crosses on the yields of the respective second parents is calculated. Selfs are usually included in this calculation. Since the yield of each cross is equal to $m+t_1+t_2+t_{12}$ where m is the general F_1 mean, t_1 and t_2 are main effects and t_{12} is the interaction, the constant-parent regression of this yield on p_2 (parental yield) is equal to the regression of (t_2+t_{12}) on p_2 and so to the sum of the regressions of t and t_{12} on p. I have already discussed the regression of the main effects t on the parents p. The k regression coefficients corresponding to the k constant-parents are sometimes statistically heterogeneous. This can be interpreted in genetic terms as a rather complicated effect of dominance and frequency

of additive genes (Griffing, 1950). For our purposes, however, such heterogeneity indicates significant constant-parent regressions of interactions on second-parent yields. This is not very surprising, for, on commonsense grounds two high-yielding parents can be expected to interact negatively rather than positively so that the interactions of crosses involving one high-yielding parent may be expected to be negatively correlated with the yield of the second parent. Since interactions are valueless for prediction anyway, such behaviour is of little interest. This does not of course discredit the constant-parent regression technique for more theoretical purposes.

6. *F_2 variances.* In the F_2, each cross was represented by two plots of ten plants each, giving 18 d.f. for " error ". Variances, when considered as statistics in their own right (rather than as indicators of the accuracy of means) are notoriously erratic, yet these figures show some interesting features. In such work it is usual to analyse log variance but in this case the use of the standard deviation is to be preferred since this is the statistic used in the estimation of upper percentiles. The results are substantially the same anyway, as may be expected. It is often argued that the differences between plants of identical genotype must be purely environmental, so that the " error " variance within different inbred lines should be constant. The tomato parents are all believed to be highly inbred commercial varieties, yet both parents and F_2 families show heterogeneity of variance (χ^2(17 d.f.) = 82·2 and χ^2(152 d.f.) = 334·8 by Bartlett's test). The average F_2 variance exceeds the average parental variance :

	Degrees of freedom	Yield Mean Square
F_2 variances . . .	2754	18·26
Parental variances . .	324	13·73

This variance ratio cannot be expected to follow Fisher's distribution exactly, since we have demonstrated heterogeneity, but it suffices to show that F_2's tend to be more variable than the parents. The F_2 standard deviations, when analysed in the usual way, themselves show " main effects " :

	Degrees of freedom	Mean Square
Main effects . . .	17	43·60
Interactions . . .	135	16·60

and these main effects are correlated with the corresponding parental standard deviations (r = 0·52, 16 d.f.). This situation can be partly—but by no means completely—explained away by the observed correlation between variance and mean yield. There is therefore some evidence that the parents transmit variability, as well as average performance, to their offspring. It is not certain whether this is purely a case of different genotypes having different error variance similar to that mentioned by Haldane (1957) quoting S. K. Roy, or whether there is also some residual heterozygosity in the " inbred " tomato varieties.

A COMPLEX GENOTYPE CONTROLLING THE PRODUCTION OF MELANOTIC TUMOURS (PSEUDOTUMOURS) IN *DROSOPHILA*

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1. INTRODUCTION

THE genetical control of the production of pseudotumours in *Drosophila* has been studied by several authors. A survey of the data known so far can be found in Barigozzi (1945) and Barigozzi and Di Pasquale (1956), who also produce evidence that the appearance of melanotic masses is controlled by several genes, called *tu*-genes (which in the stocks studied are located in the 2nd chromosome), and a complex of modifiers, probably scattered throughout the whole genotype. Castiglioni was able to demonstrate (Castiglioni and Beati, 1954; Castiglioni, 1956, 1957 and in press) that the following prerequisites are necessary for the production of melanotic masses during larval stages, in those stocks of *D. melanogaster* which are the object of the present investigation: (1) A structural change in the lymph gland, which can happen either through release of isolated cells or through detachment of entire lobes of the gland. (2) At least 2 per cent. of a special cell type, which differentiate as large cells, must be present in the hæmolymph, for the cells to congregate, clump and form melanin.

On the other hand large cell frequency and structural changes of the gland are independent since larvæ can have high numbers of large cells and an intact lymph gland.

Nothing can be said at present about the relation of gland disintegration and melanisation because no case is known where there is melanisation and at the same time an intact gland.

The aim of the present paper is to determine whether the different developmental conditions (frequency of large cells—gland transformation—melanisation) are genetically distinct.*

2. MATERIAL AND METHODS

The investigation has been carried out examining all three phenotypic traits (frequency of large cells in the larva; structure of the lymph gland in the larva; frequency of melanotic masses in the adult) in the following genotypes:

(1) Tumourless stocks: $\frac{ClB}{+}\,\frac{CyL}{Pm}\,\frac{H}{SbMe'}$, and Varese.

(2) Tumorous wild stocks: A_2, B_3, melanotic e 144 † (all spontaneous).

* We are greatly indebted to Dr M. G. Pecorini, Miss G. Bertoldini and Miss C. Halfer for valuable help in preparations of the lymph glands and counting of cells.

† We are grateful to Prof. E. Goldschmidt for sending us the stock melanotic e 144 from Israel.

(3) Homozygotes, where one or two chromosome pairs in the different possible combinations derive from $\frac{ClB}{+}\,\frac{CyL}{Pm}\,\frac{H}{SbMe'}$ and the remainder from a tumorous stock.

(4) Isogenic chr-2 recombinants derived from crosses between tumorous stocks of the type : $\frac{ClB}{+}\,\frac{tu}{tu}\,\frac{H}{SbMe'}$ and the marked stocks *b cn vg* and *cn c px*.

(5) Lines selected for *high* and for *low* production of tumours.

(6) Heterozygotes between tumorous stocks and a wild tumourless stock (Varese).

(7) Heterozygotes between different tumorous stocks.

The phenotypic data have been collected using the following techniques and methods :

(*a*) *The frequency of melanotic masses* was determined on adults. Individuals were classified into tumorous and tumourless, at a magnification of nearly 40 times.

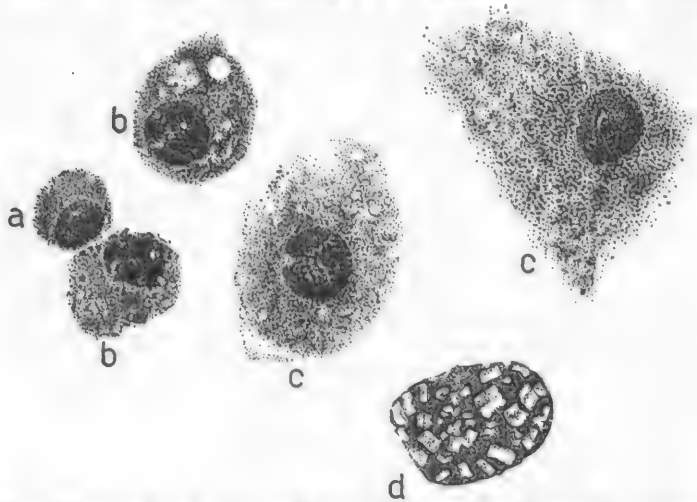

FIG. 1.—Haemolyph cells: *a*=small basophilic; *b*=midsized basophilic; *c*=large; *d*=crystalloid.

The number of masses per individual as well as their size was disregarded. For each genotype, several vials with single pairs were set up so as to reach a total offspring of several hundreds. Comparisons between frequencies were made using χ^2 * first between different cultures of the same genotype then, if homogeneous, between different genotypes.

(*b*) *Frequency of large cells* : counts were made on slides, on to which the largest possible amount of hæmolymph was put. The hæmolymph drop (from late 3rd instar larvæ) was smeared and stained with May-Grünwald Giemsa mixture. Each cell was examined under the microscope (oil immersion), and classified according to four main types (small basophilic—midsized basophilic—large—crystalloid) (fig. 1). No less than five larvæ have been used for each genotype.

* For testing homogeneity the Skory method was used ; for comparing two genotypes:

$$\chi^2 = \frac{\left(ad-bc-\frac{n}{2}\right)^2 \times n}{(a+b)(a+c)(b+d)(c+d)}.$$

Frequency comparisons between different genotypes or between tumourless and tumorous larvæ belonging to the same genotype, were made by analysis of variance of the frequencies after angular transformation. From 1000 to 3000 cells were counted in each late 3rd instar larva.

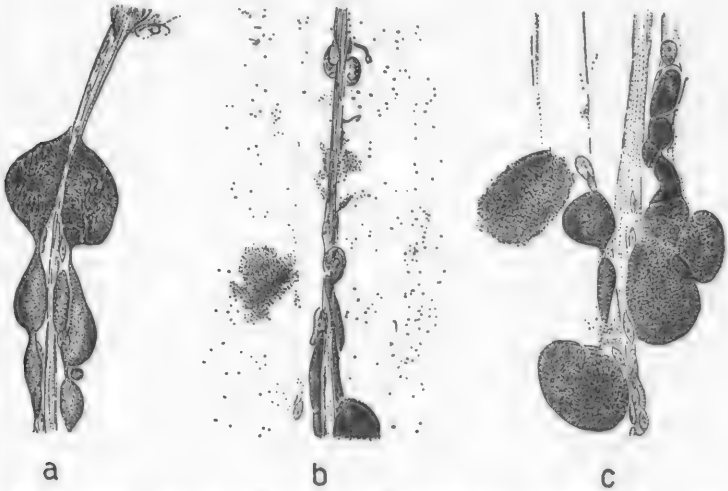

FIG. 2.—Lymph glands: *a*=intact; *b*=dissolved through release of isolated cells; *c*=dissolved through detachment of lobes.

(*c*) *The aspect and the structure of the lymph gland* were studied on preparations of whole glands, slightly coloured with Delafield Hæmatoxylin, and mounted in agar (fig. 2). Microtome sections have also been made, stained with hæmatoxylin-eosin. For each genotype 10-15 glands have been examined.

3. RESULTS

1. Table 1 shows the phenotypes of the combinations of whole chromosomes derived from the tumorous stocks, with the dominant marked chromosomes.

The table allows the following remarks: *Melanisation* is produced only when chromosome II is present.

Large cells. Comparing larvæ with and without tumours, in some cases (2 and 10) there is a significant difference in large cell frequency; in other cases (4, 8, 9) such difference is not detectable. This means that the variation of frequency between individuals in the first case is high, while in the second it is low.

Frequency of large cells is controlled by chromosomes I and II. A comparison between 1 (tumourless stock) and 5, 6, 7, proves that the replacement of the chromosome I significantly changes the frequency

in B_3 and perhaps A_2, but not in melanotic e 144. Replacing the 3rd pair (compare 1 with 11, 12, 13) changes are always insignificant.

The state of the gland. A difference between tumorous and tumourless larvæ appears in all genotypes involving A_2 and B_3: a lesser one

TABLE 1

Analysis of genotypes in different stocks and homozygous combinations

Genotypes	Percentage of tumours	Percentage of large cells		State of the gland	
		with *tu*	without *tu*	with *tu*	without *tu*
(1) $\frac{ClB}{+}\ \frac{Pm}{Cy\ L}\ \frac{H}{Sb\ Me'}$.	0·0	...	1·0	...	variable from slightly to strongly dissolved
(2) $\frac{tu\ tu\ tu}{tu\ tu\ tu}$ A_2 . . .	74·0±3·296	10·5 / $F_{1,8}=14·7$ very sign v.	1·8	dissolved	intact (basophilic fluid in the gland)
(3) $\frac{tu\ tu\ tu}{tu\ tu\ tu}$ B_3 . . .	100·0	not suitable for cells counting *	...	detachment of lobes	...
(4) $\frac{tu\ tu\ tu}{tu\ tu\ tu}$ mel e 144 .	63·8±1·770	4·6 / $F_{1,8}<1$ not sign v.	4·7	± dissolved	± intact (very small)
(5) $\frac{tu}{tu}\ \frac{Pm}{Cy\ L}\ \frac{H}{Sb\ Me'}$ A_2 .	4·9±3·330	...	4·5	...	± dissolved
(6) $\frac{tu}{tu}\ \frac{Pm}{Cy\ L}\ \frac{H}{Sb\ Me'}$ B_3 .	0·0 †	...	10·1	...	± intact
(7) $\frac{tu}{tu}\ \frac{Pm}{Cy\ L}\ \frac{H}{Sb\ Me'}$ mel e 144	0·0 †	...	1·1	...	± intact
(8) $\frac{ClB}{+}\ \frac{tu}{tu}\ \frac{H}{Sb\ Me'}$ A_2 .	60·9±4·398	3·4 / $F_{1,8}=1·16$ not sign v.	5·9	dissolved	± intact
(9) $\frac{ClB}{+}\ \frac{tu}{tu}\ \frac{H}{Sb\ Me'}$ B_3 . .	56·2±4·037	4·9 / $F_{1,8}=5·2$ not sign v.	2·8	partly dissolved with lobes detachment	± intact
(10) $\frac{ClB}{+}\ \frac{tu}{tu}\ \frac{H}{Sb\ Me'}$ mel e 144	10·7±2·451	5·7 / $F_{1,8}=74·0$ very sign v.	1·9	± dissolved	less dissolved (very small)
(11) $\frac{ClB}{+}\ \frac{Pm}{Cy\ L}\ \frac{tu}{tu}$ A^2 . .	0·0 †	...	0·1	...	intact
(12) $\frac{ClB}{+}\ \frac{Pm}{Cy\ L}\ \frac{tu}{tu}$ B_3 . .	0·0 †	...	1·7	...	intact
(13) $\frac{ClB}{+}\ \frac{Pm}{Cy\ L}\ \frac{tu}{tu}$ mel e 144 .	0·0 †	...	1·5	...	intact

* Cells remain for the most part within the gland.

† All zeros reported have actually been found in countings: nonetheless other data of the same genotypes may also give very low percentages.

results in those involving melanotic e 144. This proves that the tumour frequency in melanotic e 144 is also due to a variable action of the melanisation genes. In fact, both the other elements are very little variable in the stock (see p. 157).

The state of the gland is thus controlled by all three major chromosomes. A comparison between 1 and 5, 6, 7 proves that a slight change occurs when the 1st pair is replaced. Similarly, when a replacement concerns the 2nd, a change occurs (compare 1 with 8, 9, 10). An

TABLE 2

Phenotype of tumourless recombinants

Genotype	Percentage of tumours	Percentage of large cells	State of the gland
$\frac{ClB \text{ recomb. } A_2}{+ \text{ recomb. } A_2} \frac{H}{Sb\ Me'}$ . .	0·0	11·0	± intact
$\frac{ClB \text{ recomb. } B_3}{+ \text{ recomb. } B_3} \frac{H}{Sb\ Me'}$ . .	0·0	23·8	± intact

effect is also produced by the 3rd chromosome (compare 1 with 11, 12, 13).

2. Chromosome II has been submitted to a more detailed analysis. Chromosomes derived from females heterozygous for chromosome II

TABLE 3

Tumour manifestation in crosses between recombinants having the same phenotype cn c px tu

Percentage of tumours in the offspring	No. of crosses
0	6
1-10	19
11-20	2
21	2

Percentage of tumours			No. of flies
Parent lines		Offspring	
♂	♀		
8·6	63·3	0·0	102
63·3	11·1	0·0	78
9·3	4·8	0·0	96
9·3	2·6	0·0	94
9·3	8·6	0·0	252
8·6	4·8	0·0	41

of A_2 or B_3 and *b cn vg* were made isogenic. Chromosomes I and III being *ClB*/+ and *H*/*SbMe'*. The *b cn vg* stock used has a tumour manifestation of about 3 per cent., a percentage of large cells of 2·1 and a more or less intact gland. Some of the isogenic 2nd chromosome

stocks so obtained were tumour free and were submitted to the usual examination for large cells and lymph gland (see table 2).

In these recombinants the cell frequency is higher than the figure recorded on table 1 for non-recombinants $\left(\frac{ClB}{+}\frac{tu}{tu}\frac{H}{SbMe'}\right)$ in both A_2 and B_3 but too much reliance should not be placed on this comparison as the data in tables 1 and 2 were obtained at different times.

Recombinants were also obtained between B_3 and *cn c px*. In this case no analysis of the phenotype was made, except for classifying the adults into tumorous and non-tumorous, marked and wild.

A number of isogenic stocks all showing the same phenotype *cn c px tu* were intercrossed, and the results from 29 crosses are given in table 3*a*.

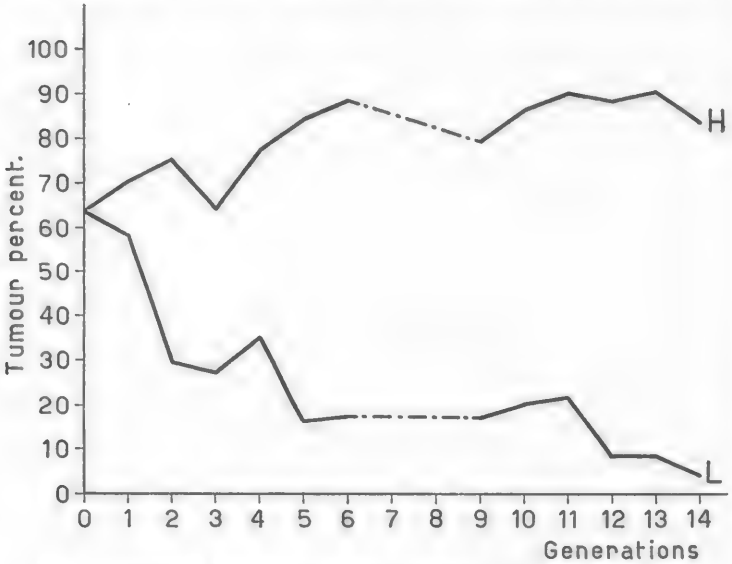

FIG. 3.—Selection for and against tnmours in melanotic e 144: *H*=high; *L*=low.

Table 3*b* gives further details of the 6 crosses which have tumour-free progeny.

It appears from table 3 that the "factor" controlling melanisation can be split through recombination into a number of non-allelic parts. In other words, the melanising "gene" *tu*, is in fact a group of loci (or a complex locus), or a system of polygenes crowded in a portion of the right arm of the 2nd chromosome (see Barigozzi and Di Pasquale, 1956).

3. Selection experiments for and against tumours have been made on melanotic e 144. Family selection was used; in each generation *ten* cultures were set up and the progeny showing the highest or lowest

incidence of tumour in the respective lines was used to set up the next generation. This selection pressure of 10 per cent. was applied for 12 generations.* The results are given in table 4 and fig. 3.

TABLE 4

Selection for and against tumours in melanotic e 144

e 144 melanotic		Percentage of tumours	Percentage of large cells		State of the gland	
			with *tu*	without *tu*	with *tu*	without *tu*
before selection		$63{\cdot}8 \pm 1{\cdot}770$	4·6	4·7	± dissolved	± intact (very small)
			$F_{1,\ 8} < 1$ not sign.			
after selection	H	$83{\cdot}5 \pm 3{\cdot}555$	2·3	0·8	frequently dissolved	± dissolved
			$F_{1,\ 8} = 1{\cdot}6$ not sign.			
			$F_{1,\ 18} = 2{\cdot}8$ not sign.			
	L	$4{\cdot}1 \pm 1{\cdot}264$	5·0	2·1	frequently dissolved	± intact (very small)
			$F_{1,\ 8} < 1$ not sign.			

H = high. L = low.

In the 12th selected generation H and L lines were crossed in both directions, and the following results obtained (table 5).

TABLE 5

Crosses between selected lines (H, L) of melanotic e 144

Crosses	Percentage of tumours		Percentage of large cells		State of the gland
			with *tu*	without *tu*	
♀ H × ♂ L	$56{\cdot}2 \pm 4{\cdot}106$	$\chi^2_1 = 2{\cdot}75$ not sign.	2·8	0·7	± dissolved
			$F_{1,\ 8} = 3{\cdot}2$ not sign.		
			$F_{1,\ 18} < 1$ not sign.		
♀ L × ♂ H	$66{\cdot}4 \pm 3{\cdot}991$		1·3	2·4	
			$F_{1,\ 8} < 1$ not sign.		

The conclusions are that: (1) selection has a clear effect on melanisation, a less clear effect on the state of the gland, and no recognisable one in the frequency of large cells. The three phenomena behave, thus, as largely independent; (2) the cross between H and L gives

* The lines were actually bred for 14 generations, but selection was not carried out in the 7th and 8th generations.

practically the same result in both reciprocal crosses and in respect to tumour frequency approximate to that of the stock before selection.

4. The genetical relationship between tumorous and non-tumorous stocks has also been studied in crosses with Varese. All three characters were recorded in F_1 individuals and the results are given in table 6. Except where indicated, no sex difference was observed.

TABLE 6

Crosses between tumorous and non-tumorous stocks (percentage of large cells and state of gland in tumourless individuals)

Genotypes (cytoplasm Varese)	Percentage of tumours	Percentage of large cells	State of gland
(1) Varese	0·0		intact
(2) $\frac{B_3}{+}\frac{B_3}{+}\frac{B_3}{+}$	1·6±0·598		intact
(3) $\frac{mel}{+}\frac{mel}{+}\frac{mel}{+}$	0·0	$F_{3,16} = 2·4$ not sign.	intact
(4) $\frac{A_2}{+}\frac{A_2}{+}\frac{A_2}{+}$	2·7±0·799		intact (basophilic fluid)
(5) $\frac{A_2}{+}\frac{A_2}{+}\frac{H \text{ or } Sb\ Me'}{+}$ ♂ / ♀	0·7±0·717 / 8·9±2·557 $\chi_1^2 = 7·91$ sign.	$F_{1,4} = 11·7$ sign.	nearly intact
(6) $\frac{ClB}{+}\frac{A_2}{+}\frac{H \text{ or } Sb\ Me'}{+}$ ♂ / ♀	0·0 / 4·7±1·527	$F_{1,4} = 3·3$ not sign.	± intact

Note: No difference has been found in reciprocal crosses except in 5 and 6.

We can conclude that: (1) all tumorous stocks behave as nearly completely recessive against Varese (tumourless) with reference to all three characters considered; (2) a peculiar trait of A_2, *i.e* the presence of a basophilic fluid within the gland, manifests itself in the heterozygote; (3) in $A_2/+$ heterozygotes for one single chromosome and for two chromosomes (5, 6) there is a significant difference between the sexes, with respect to the tumour frequency and (in 5 only) with respect to the frequency of the large cells as well. This proves an interaction between X and 3rd chromosome, because in 4 (where there is $\frac{A_2}{+}$ 3rd, instead of $\frac{+}{Sb\ Me' \text{ or } H}$) the difference between sexes disappears; (4) the state of the gland, as well as the cell frequency, do not change greatly in the different crosses.

5. The genetical relationship between the tumorous stocks have also been investigated. The results are summarised in table 7.

The conclusions are: (1) Heterozygosity produces a decrease in tumour frequency. Since this gives information about the genotypic similarity between stocks, we could arrive at this serial classification (the closer ones are the most similar): B_3-A_2-melanotic e 144. All three stocks behave as giving a more or less intermediate manifestation in heterozygotes.

(2) Heterozygosity also causes a decrease to a very low level of the frequency of large cells in $A_2 \times B_3$ heterozygotes. No reduction (or even an increase) is produced by the presence of melanotic e 144. The same is true of the heterozygotes for only the 2nd chromosome.

TABLE 7

Crosses between different tumorous stocks (percentage of large cells and state of gland in tumourless individuals)

Genotypes	Percentage of tumours	Percentage of large cells	State of the gland
♀ A_2			
(1) $\frac{A_2\ A_2\ A_2}{B_3\ B_3\ B_3}$	15·2±1·594	2·1	intact and swollen
(2) $\frac{ClB\ A_2\ H}{+\ B_3\ Sb\ Me'}$	6·0±1·273	1·8	± intact
	$\chi_1^2 = 7{\cdot}05$ sign.		
♀ B_3			
(3) $\frac{A_2\ A_2\ A_2}{B_3\ B_3\ B_3}$	8·0±1·262	1·4	intact and swollen
(4) $\frac{ClB\ A_2\ H}{+\ B_3\ Sb\ Me'}$	7·1±1·898	2·6	± intact
	$\chi_1^2 = 0{\cdot}05$ not sign.		
♀ mel e 144			
(5) $\frac{A_2\ A_2\ A_2}{mel\ mel\ mel}$	11·9±2·380	11·0	variable (± intact)
(6) $\frac{ClB\ A_2\ H}{+\ mel\ Sb\ Me'}$	*18·9*±2·327	8·7	variable (often dissolved)
♀ A_2			
(7) $\frac{A_2\ A_2\ A_2}{mel\ mel\ mel}$	22·7±3·079	17·9	variable (± intact)
(8) $\frac{ClB\ A_2\ H}{+\ mel\ Sb\ Me'}$	14·5±1·956	6·3	variable (often dissolved)
♀ *mel.e* 144			
(9) $\frac{B_3\ B_3\ B_3}{mel\ mel\ mel}$	5·4±1·318	5·5	intact
(10) $\frac{ClB\ B_3\ H}{+\ mel\ Sb\ Me'}$	7·1±1·534	8·1	variable (often dissolved)
♀ B_3			
(11) $\frac{B_3\ B_3\ B_3}{mel\ mel\ mel}$	*3·4*±0·653	7·6	± intact
(12) $\frac{ClB\ B_3\ H}{+\ mel\ Sb\ Me'}$	7·0±1·442	2·5	variable (often dissolved)

Note: Italic figures refer to heterogeneous samples, thus unsuitable for further statistical elaboration.

(3) Heterozygosity between A_2 and B_3 induces a gland structure which resembles that of tumourless individuals of A_2; melanotic e 144 introduces a variability, which leads to an often rather high gland dissolution thus showing once more that gland dissolution is not sufficient to produce melanisation.

(4) The three elements acting on tumour production behave rather differently in the three stocks.

(5) Where comparisons are possible (1, 2, 3, 4), the cytoplasm can exert an effect on the tumour percentage: more precisely when A_2 cytoplasm is present, it increases the tumour frequency in $A_2 \times B_3$ heterozygotes for all chromosomes, whereas heterozygotes for the 2nd pair only do not show this effect. Comparisons with heterozygotes carrying melanotic e 144 are impossible, because of the heterogeneity of the samples.

4. GENERAL CONCLUSIONS AND DISCUSSION

(1) In three spontaneous stocks of *D. melanogaster*, the production of pseudotumour is the result of three developmental steps (detachment of cells from the lymph gland; presence of large cells in the hæmolymph in a frequency of no less than 2 per cent.; melanisation of clumps of large cells).

(2) The first two steps are not strictly dependent in development, because it is possible to obtain tumourless genotypes with high frequency of large cells and intact gland (see table 2). Melanisation is conditioned by high frequency of large cells in the hæmolymph and a structural change in the gland as previously described. In all genotypes analysed, the process in tumorous individuals is constant, except for the mode of the displacement of the cells from the lymph gland.

(3) All chromosomes are active in controlling the production of tumours. The replacement of whole chromosomes, recombination and heterozygosity permit us, however, to distinguish distinct functions in the three main chromosome pairs: (a) *melanisation*, is controlled nearly exclusively by the 2nd chromosome (*tu*-genes), where several loci are concentrated in small portions, which can be different in different stocks. These genes behave as nearly recessive, if tested against " no melanisation ", but are moderately effective in heterozygotes between tumorous stocks in spite of their different location; (b) *frequency of large cells*, is controlled at least by the 1st and the 2nd. The 2nd pair controls this character, as well as melanisation; no elements are known concerning the 3rd pair. These genes behave as recessive in heterozygotes; (c) *state of the gland*, is controlled by genes located also on all major chromosomes. Alleles producing changes in the lymph gland during larval stages behave as recessive as compared with those keeping it intact. A typical swelling is produced in heterozygotes between A_2 and B_3.

Melanisation is genetically independent of the other two phenomena in melanotic e 144, as proved by selection experiments.

(4) The phenotype is the result of the integration of the action exerted by factors distributed unevenly (at least partially) along all major chromosomes. The melanotic tumour is, in other words, the phenotype corresponding to at least three different parts of the genome working together, each having a typical and recognisable effect.

These findings can now be shortly discussed with respect to the main problems concerning the heredity of the quantitative characters.

A character is called quantitative when it can be described by means of measurements; this is true independently of whether it is controlled by oligogenes or by polygenes. The presence of the melanotic spots in *Drosophila* can be considered as a quantitative character, because, taking into consideration the incidence of the trait, the stocks can be classified into a series, going from tumour incidence of 0 up to 100 per cent., including all intermediate degrees.

It should be noted that the recognition of tumours depends on the technique used (magnification, examination of sections, etc.), and stocks classified as non-tumorous at an inspection with low magnification, might reveal the presence of some very slight melanisation, when examined using more refined means of observation. Quantitative characters controlled by several genes (polygenes) are intermediate in heredity: this is not found in melanotic tumours, which behave, in the majority of the stocks studied so far, as nearly completely recessive or, rarely, as dominant. This fact seems to establish a difference between this character and other well studied examples of quantitative characters (abdominal chaetæ in *Drosophila*, Mather, 1949). The polygenic characters have in common another property: genes are able to work together irrespective of their location, so that the final result is more or less influenced by many or by all portions of the chromosomes. This phenomenon is recognisable in melanotic tumours, because these appear with considerable incidence in crosses between tumorous stocks, although not so frequently as in the stocks. This fact favours the view that a very diffuse physiological affinity exists between distant loci, especially in stocks A_2 and B_3, where *tu*-genes are located at the opposite ends of the 2nd chromosome (Barigozzi and Di Pasquale, 1956).

Thus, the inheritance of the presence of melanotic masses, when compared with that of conventional polygenic characters, seems to occupy a special position, giving to *tu*-genes an intermediate position between oligogenes and polygenes.

What we have remarked about the production of melanotic masses, can be extended to both the prerequisites for their formation, *i.e.* the frequency of the large cells, and the behaviour of the gland. Both characters are controlled by several factors; at least three, because all three major chromosomes are involved. However, high cell frequency is recessive to low cell frequency, and dissolved gland to intact gland. Unfortunately we do not know where the factors controlling the blood cells and the lymph gland state are located along the chromosome; nothing more can be said about their behaviour in compounds.

Summarising, the production of pseudotumours forms the most complex character so far analysed in *Drosophila*, being brought about by at least three elementary traits, and at least three systems of factors.

5. SUMMARY

1. The complicated mechanism of tumour production, already known in several tumorous stocks of *D. melanogaster*, was submitted to genetic analysis in three stocks, in order to prove whether the different developmental steps are controlled by the same or by different genes. Using different genetical techniques, evidence was found that different systems of factors are working.

2. These systems act during the larval stages. One of them controls the frequency of the hæmolymph cells; a second the behaviour of the lymph gland; a third the production of melanin by the large cells of the hæmolymph. The first and second systems are multichromosomal, while the third is localised in the 2nd chromosome, and, in some stocks, in one restricted section of it.

3. In no case was it demonstrated that the same genes act on the different developmental steps, listed above. So the authors incline to believe that pleiotropy is not demonstrated.

4. The genes for melanisation (*tu*-genes), which are more suitable for investigation, have some characters in common with typical polygenes (several loci with identical or similar effect, physiological allelism between groups located in different parts of the chromosome); their recessiveness and restricted location in the genome, on the one hand, and technical reasons on the other, let them appear as controlling a discontinuous character.

6. REFERENCES

BARIGOZZI, C. 1954. The appearance of abnormal groups of cells during or after development: tumors and pseudotumors. *Proc. IX Int. Congress of Genetics*, 1953 (Bellagio) *Caryologia, 6* (suppl.), 338-354.

BARIGOZZI, C. 1958. Melanotic Tumours in *Drosophila* *J. cell. comp. Physiology, 52*, (suppl.), 371-381.

BARIGOZZI, C., AND DI PASQUALE, A. 1956. A contribution to the genetics of the so-called melanotic tumors (pseudotumors) of *Drosophila melanogaster*. *Rend. Ist. Lomb. Sci. Lett., 90*, 484-509.

CASTIGLIONI, M. C. 1956. Nuove osservazioni sulla produzione degli pseudotumori in *Drosophila melanogaster*. "*Atti II Riunione AGI*," *La Ricerca Scientifica* suppl. *26*, 125-130.

CASTIGLIONI, M. C. 1957. Le cellule dell'emolinfa di *Drosophila melanogaster* in relazione al genotipo e alla produzione degli pseudotumori. "*Atti III Riunione AGI*," *La Ricerca Scientifica*, suppl. *27*, 51-58.

CASTIGLIONI, M. C., AND BEATI, G. 1954. Production of pseudotumors in Drosophila, after injection of hæmolymph. *Experientia, 10*, 501-502.

MATHER, K. 1949. *Biometrical Genetics*. Methuen, London.

THE EVOLUTION OF MIMICRY IN THE BUTTERFLY *PAPILIO DARDANUS*

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1. INTRODUCTION

When Bates put forward the mimicry hypothesis which bears his name, Darwin (1872), although accepting it, had some difficulty in explaining the evolution of the mimetic resemblance of several distinct species to one distasteful model by a series of small changes, a requirement of his general theory of evolution. He said " it is necessary to suppose in some cases that ancient members belonging to several distinct groups, before they had diverged to their present extent, accidentally resembled a member of another and protected group in a sufficient degree to afford some slight protection; this having given the basis for the subsequent acquisition of the most perfect resemblance ". Punnett (1915) realised that the difficulty is even more acute when one is dealing with a polymorphic species whose forms mimic very distantly related models. Knowing that, in those butterflies which had been investigated genetically, the forms differed by single allelomorphs he concluded that the mimicry did not evolve gradually and did not confer any advantage or disadvantage to the individual. He argued that an allelomorph arises at a single step by mutation and that therefore the mimicry also arises by chance at a single step. Goldschmidt (1945) although not denying that mimicry confers some advantage to its possessors also maintained that the resemblance arises fully perfected by a single mutation of a gene distinct from that producing the colour pattern in the model, but producing a similar effect in the mimic. Fisher and Ford (see Fisher, 1930; Carpenter and Ford, 1933; Ford, 1953) on the other hand have put forward a view intermediate between the extreme ones of Darwin on the one hand and Goldschmidt on the other. They maintain that a resemblance between the mimic and model as good as that often found in nature cannot reasonably be expected to arise by chance. They therefore suggest that a mutant becomes established which gives a sufficient resemblance to be advantageous and that this is gradually improved by the selection of modifiers which alter the effects produced by the mutant.

Ford (1953) has raised serious theoretical objections to Goldschmidt's (1945) hypothesis and he pointed out how the validity of each hypothesis can be tested. He notes that a particular mimic usually varies in appearance in parallel with the local race of its model and that on his own hypothesis one would expect these allopatric

mimetic forms of one model to differ multifactorially. However, if mimicry arises by mutation and is not thereafter modified, as is postulated by Goldschmidt, one would expect such local variation in the mimic to be controlled by multiple allelomorphs as Goldschmidt himself predicts. In 1954 we decided to investigate this problem genetically.

2. MATERIAL AND METHODS

The work has been carried out in two stages. Firstly, it was necessary to understand the mode of inheritance of various sympatric varieties and secondly, to make suitable race crosses to test the two hypotheses. Ford (1953) suggested that the best material for such a study might be the butterfly *Hypolimnas dubius* since both sexes

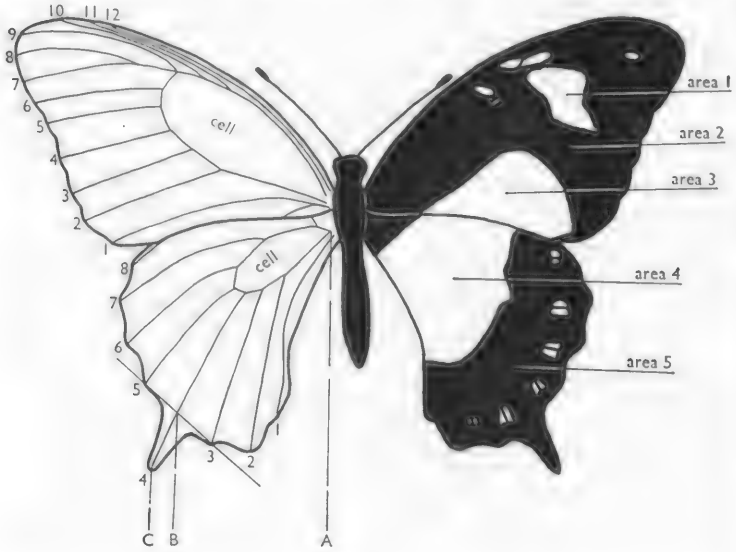

FIG. 1

are polymorphic and mimetic and the genetic status of the forms is relatively well known. However, we decided to use the African butterfly *Papilio dardanus* Brown. Although it suffers from the disadvantages that it is only polymorphic and mimetic in the female and has an almost bewildering variety of forms, whose genetics were unknown at the time, it had the overwhelming advantages that (*a*) it can be hand-mated, (*b*) it will lay eggs readily in captivity, (*c*) it has a larva one of whose food plants, *Citrus*, was known to be available in England, (*d*) the butterfly is widely distributed and common over much of Africa and (*e*) it has a large number of relatively distinct races whose distribution has been worked out in detail.

A description of the races and forms can be obtained from the excellent paper by Ford (1936) and the standard works he cites. An account of the forms we have investigated can be obtained from Clarke and Sheppard (1960). The pattern differences between the forms can be described in terms of the numbered areas, and the length of the tails, if present, can be measured by the length BC and that of the wing BA (fig. 1). The method of determining these points and of adjusting

tail length to take into account variations in the size of the wing itself are given by Clarke and Sheppard (1960).

The details of the breeding techniques are given by Clarke and Sheppard (1959*a*) and an account of the genetics of most of the major varieties by Ford (1936) and Clarke and Sheppard (1959*b*, and in preparation). The *hippocoonides*, *cenea*, *natalica*, *trophonius*, *leighi*, pale-*poultoni* and the yellow male-like non-mimetic forms of the female are controlled by a multiple allelomorphic series or by closely linked genes whose effects are sex-controlled. Bright-*poultoni*, *niobe* and *planemoides* are determined unifactorially by genes whose effects are sex-controlled and they are probably members of the same allelomorphic series as all the others. The absence of tails in the females is determined by a single locus not linked to the multiple-allelomorphic series and its effect is also sex-controlled.

3. RESULTS

(1) *Modification of the* hippocoonides *pattern*

The form *hippocoonides* is a black and white insect found in South and East Africa (races *cenea*, *tibullus* and *polytrophus*) which mimics *Amauris niavius dominicanus* (plate 1, no. 1). The mimic has a narrow black border to the hindwing (area 5) with small white spots on it near its distal edge. The proximal edge of this black area (situated $\frac{1}{2}$ to $\frac{3}{4}$ of the way from the margin of the wing to the cell) is fairly distinct in outline and has short black rays extending from it towards the cell, and situated parallel to and midway between the veins of the wing (plate 1, no. 3).

In West and Central Africa (race *dardanus*) there is an allopatric form *hippocoon* which mimics *A. n. niavius* the western race of the model of *hippocoonides*. The mimic like the model differs from the eastern form in the amount of black on the hindwing (area 5). Area 4 is much reduced in size since the black of area 5 extends to the cell or nearly to it. The white spots near the margin of the wing are much reduced in size or absent and the proximal edge of area 5 is far less distinct than in *hippocoonides* since it shades off gradually into the white area owing to a liberal scattering of black scales along the border. The rays are also extended in length and end near the cell (plate 1, no. 4). All these characters are concerned in the mimetic resemblance since all enhance the black appearance of the hindwing, a characteristic of the western model (plate 1, no. 2).

On crossing a *hippocoon* stock with *hippocoonides* from South Africa an F_1 generation is produced which normally is much like the less extreme *hippocoon* forms in appearance. That is to say, although the individuals have a broad black border to the hindwing it is usually slightly narrower than the average for *hippocoon* since it does not usually reach the cell, the black rays are slightly shorter and there is a greater tendency for the white spots near the margin of the hindwing to appear. However, in two F_1 families out of three, using the west coast (Ghana) *hippocoon*, the F_1 were *hippocoonides*-like in appearance (one insect excepted) but not in thirteen families using the Uganda stock of *dardanus*. Thus there is some evidence that the genetic control

of the difference between *hippocoon* and *hippocoonides* is not the same in all individuals.

In the F_2 and first backcross to *hippocoonides* of the *hippocoon*-like F_1 there is no clear cut segregation into *hippocoonides*-, *hippocoon*- or F_1-like individuals. Not only are all grades of intermediates found but the various characters which determine the difference between *hippocoon* and *hippocoonides* segregate and are themselves variable in expression. Thus one can have a narrow black border (area 5) with marked rays (plate 2, no. 8), with no rays (no. 7), a broad border with long rays (no. 6), with short rays, and also independent assortment of the expression of the white spotting near the margin of the wing (nos. 4-8).

We are therefore led to the conclusion that *hippocoon* and *hippocoonides* do not differ by a pair of allelomorphs but by a minimum of three and probably many more than three loci.

In race *antinorii* from Abyssinia there is a black and white form *niavioides* which mimics *Amauris niavius* but is a much poorer mimic than either *hippocoon* or *hippocoonides*. It has the normal pattern of the mimic on the forewing but not only does the hindwing have tails (see below) but the black border (area 5) is far more irregular in outline than in the model or the other two forms and the white spots near the margin of the wing tend to be far larger and less well defined in shape. The F_1 hybrid between *niavioides* and *hippocoonides* has a hindwing border which is more regular in outline and gives a better mimetic pattern. In the F_2 there is considerable variability but no clear cut segregation into *niavioides*-, *hippocoonides*- and F_1-like individuals. In fact, of 31 insects examined none were of the typical *niavioides* form. Thus here again we must conclude that *hippocoonides* and *niavioides* differ by more than one pair of allelomorphs.

(ii) *Modification of the* cenea *pattern*

The form *cenea*, a good mimic of *Amauris echeria* and *A. albimaculata* (plate 1, nos. 8 and 6), is found in South and East Africa as far west as the north shore of Lake Victoria (race *dardanus*). It is not found in West Africa nor are its models. The variety is dominant to *hippocoonides*, and to *hippocoon* taken from the area of Lake Victoria. The *cenea*/*hippocoon* heterozygotes differ slightly from the South African forms (plate 1, no. 8, and plate 2, no. 1) and are like those found in the vicinity of Lake Victoria and in Kenya (race *polytrophus*). The difference involves the white spots in area 1 which are variable in size, often being slightly enlarged, or, in a small minority of wild individuals and of our heterozygotes, being actually joined up to form a white patch of variable size. Thus in race *dardanus*, from an area where *cenea* is uncommon (7 per cent. or less of the population) and from Kenya (race *polytrophus*) where the models are rare, the mimics are more variable and slightly less perfect in their resemblance. In contrast to this, the only two families we have so far obtained in which

the *cenea/hippocoon* heterozygote has been produced using *dardanus* from West Africa (Ghana) the white spots of area 1 of the forewing were absent and replaced by a large white patch similar to that of *hippocoon*. Thus all these insects resembled the most extreme forms of the eastern hybrids. Moreover unlike the latter the black border of the hindwing (area 5) is very much broader, actually reaching the cell (plate 2, no. 2). Our data as far as they go suggest that there is considerable variability in the gene-complex of race *dardanus* with respect to modifiers affecting the expression of *cenea*. In the east there are modifiers giving quite good mimicry but not as perfect as in South Africa where the mimic is common. In the west, however, these modifiers appear to be absent or much rarer and consequently the mimicry is far less perfect.

(iii) *Modification of the* trophonius *pattern*

The form *trophonius* is found throughout South, East and West Africa and it mimics *Danaus chrysippus* (plate 1, nos. 5 and 7). It has the same black pattern as *hippocoonides* but areas 3 and 4 are orange-brown not white. In both the east and the west it has a narrow black border in area 5, thus conforming to the pattern of the model, but in the west the black rays between the veins are elongated and the form is called *trophonissa* by entomologists. It is dominant to *hippocoonides* and *hippocoon* and produces normal mimicry in hybrids between South African *trophonius* and both eastern (Uganda) and western (Ghana) *hippocoon* (plate 2, no. 3).

The only difference between the hybrids and South African *trophonius* is that the rays are sometimes rather longer in the hybrid thus conforming to the *trophonissa* pattern. It seems that in contrast to the situation found with *cenea* the *dardanus* race does not vary much with respect to modifiers affecting the mimicry of *trophonius*, a form found throughout its area of distribution.

(iv) *Modification of the* planemoides *pattern*

The mimic *planemoides* resembles *Bematistes poggei*, the male of *B. macarista* and perhaps the female form *aurivillii* of *Acraea alciope*. It is found commonly in Central Africa where its models are common but elsewhere only as a rarity often with a rather incomplete development of the mimetic pattern (proto-*planemoides*). The form, which is dominant to *hippocoon*, has hindwings which are white and have a broad black border (area 5) similar to that in *hippocoon* but with the white spots, if present, replaced by orange-brown. The forewings are in the main black but there is a broad irregularly shaped bar of orange-brown pigment covering area 1, area 2, and the distal half of area 3.

On crossing *planemoides* from Entebbe with Kenya stock of the race *polytrophus*, *planemoides/hippocoonides* heterozygotes were obtained. These had the general coloration and pattern of *planemoides* except that the

orange-brown area on the hindwings was divided by a broad black bar covering area 2. Thus the mimetic pattern became imperfect when part of the gene-complex of *polytrophus* was introduced into the genetic constitution of the heterozygotes. These imperfect mimics were similar to many taken from wild populations or bred from wild females of the *polytrophus* race. One insect similar to our hybrids is figured in colour by Ford (1936, plate 4, no. 3). The results indicate that in the gene-complex of *polytrophus* from Kenya, where the models are rare or absent, the mimetic pattern of *planemoides* partially breaks down.

(v) *Hybridisation with the Abyssinian and Madagascan races*

Besides the crosses between races of the main African stock, hybrids have also been obtained between some of the mimetic forms of Central and South Africa and the isolated races *meriones* from Madagascar and *antinorii* from Abyssinia. The results have been reported by Clarke and Sheppard (1960) but a summary of them will be given here.

By far the commonest female form in the Abyssinian race is black and yellow and looks much like the male. This form accounts for 80 per cent. and probably more of the female forms and has tails like all the other Abyssinian females. This contrasts with the other races we have considered in which the females are always tailless. The next commonest form in race *antinorii* is *niavioides* which as we have seen is a modified *hippocoonides*. Besides this form there is a much rarer mimic of *D. chrysippus* called *ruspinae*, looking just like a tailed *trophonius*, and a tailed *cenea*, which has only been reported twice, mimicking *A. echeria*. Both *niavioides* and "*cenea*" are completely recessive to the yellow male-like form (the presumed primitive pattern) and *cenea* is dominant to *niavioides*. The gene controlling the *trophonius* pattern is also dominant in its effect to *niavioides* and produces a good mimic of *D. chrysippus*. However, the hybrid F_1 heterozygote between the yellow form and *trophonius* produces a confused result. The colour pattern of the hindwings is like *trophonius*, but although area 3 is orange-brown in colour, area 2 is yellow not black and area 1 shows varying amounts of yellow and orange-brown pigment. Thus the mimicry has completely broken down in this hybrid genotype. Since no insect resembling this heterozygote has ever been seen in the wild, despite the fact that several *ruspinae* have been caught, we must come to one of two conclusions; either (*a*) on replacing the hybrid gene-complex by a pure *antinorii* one, this intermediate pattern would vanish and mimicry would be restored, or (*b*) *trophonius* does not occur in race *antinorii* and *ruspinae* is controlled by a different gene, the *antinorii* gene-complex being adjusted to the latter, but not to *trophonius*.

The race *meriones* is monomorphic, all the females being like the males and tailed. In the F_1 heterozygotes between this race and the forms *cenea* and *hippocoonides* the mimicry breaks down. The pattern

of the *hippocoonides* heterozygote is rather like that of *niavioides* except that the pale areas are yellow, but a paler yellow than in *meriones* itself. On backcrossing into the South African race the black pattern becomes identical with *hippocoonides* but the pale areas remain pale yellow. Insects indistinguishable from this have been produced among the second, third and fourth backcross generations of yellow insects, derived from Abyssinia to Central African stock. It therefore seems beyond dispute that the South and Central African races possess modifiers which materially increase the mimetic resemblance between the yellow/*hippocoonides* heterozygotes and the model *Amauris niavius dominicanus* (for plate see Clarke and Sheppard, 1960).

The *cenea*/yellow heterozygote is quite unlike *cenea* and is very similar to the *hippocoonides*/yellow F_1 except that the yellow is more orange and area 1 shows a tendency to break up into yellow spots surrounded by black.

The *trophonius*/yellow heterozygote has an appearance very different from the similar hybrid with race *antinorii*. However, this depends chiefly on the fact that area 2 is black not yellow. The amount of orange-brown pigment in area 1 is very variable in both these hybrids. On one specimen, area 1 is orange-brown but on another it is of the palest yellow. Moreover in the latter individual the black bar area 2 is reduced and there is pale yellow pigment next to it in area 3. Thus the insect is an almost exact replica of proto-*trophonius* an imperfect mimic found in race *polytrophus*.

(vi) *The inheritance of tails*

In the Madagascan race the tails of the females (mean 14.8 mm.) are as long or longer than those of the males (mean 14·4 mm.). The tailless condition is controlled by a single allelomorph (*T*) which shows no dominance, the heterozygotes of the F_1 generation having an intermediate tail length and considerable variability from individual to individual (mean 4·4 mm.). The tailed insects (*Tt*) of the first backcross to South African stock have rather shorter tails than most of the F_1 heterozygotes suggesting that the South African race has modifiers shortening tail length in the heterozygote.

In the similar *antinorii* F_1 hybrid heterozygotes and the first backcross to race *antinorii* the heterozygotes have a shorter mean tail length than in the previous Madagascan hybrid (mean 1·9 mm.) and about half the individuals appear to be tailless, so that this condition is dominant in many individuals. On examining the pure *antinorii* females we find that the mean tail length of the yellow females (12·5 mm.) is less than that of the males (14·2 mm.; $P<0.001$) and the variance is greater ($P<0.001$) as it is also compared with that of the Madagascan males ($P<0.001$) or females ($P<0.001$). The mean tail length of the mimetic *antinorii* females (9·0 mm.) is even less than in the yellow ones ($P<0.001$) and there is a suggestion that the variance is

greater. These findings also apply to the tailed (*tt*) individuals of the first backcross to *antinorii* (difference of means $P<0{\cdot}001$, of variance $P<0{\cdot}05$) (see Clarke and Sheppard, 1960). Thus the monomorphic *meriones* and polymorphic *antinorii* races differ with respect to modifiers affecting tail length.

4. DISCUSSION

Races *antinorii* and *meriones* are particularly pertinent to any discussion on the ability of disruptive selection (Mather, 1955) gradually to alter a mimic towards a more perfect resemblance. It is certain that the absence of tails increases the resemblance between model and mimic to a quite remarkable degree. Now judging by the greater mean length of the tails of *meriones* females as compared with males and the small variance in them and in the males of all races (see Clarke and Sheppard, 1960), we may conclude with a fair degree of confidence that the tailed condition is advantageous in the yellow non-mimetic pattern. If this be so, in the polymorphic Abyssinian race where the allelomorph *T* for the absence of tails is not found, one would expect disruptive selection and the consequent accumulation of modifiers reducing tail length in the mimics. Moreover, since Thoday (1959) has shown that disruptive selection leads to increased variance within the population, one would also expect the female variance, particularly that of the mimetic forms, to be greater in the *antinorii* than in the monomorphic *meriones* race.

The data from wild caught specimens show that in *antinorii* the females have a shorter mean tail length than the males and that this is particularly marked in the mimetic forms. The first backcross to *antinorii* of the F_1 hybrid with Central African stock is in agreement with respect to the shorter mean tail length of the mimetic females. Moreover, as predicted, the variance is greater in the females, particularly the mimetic ones, than in the males of any race or of the *meriones* females.

Now it might be argued that the allelomorphs for mimetic patterns were themselves affecting tail length thus reducing the mean below that of the yellow females. But the effect of a gene cannot be taken in isolation from the particular gene-complex in which the allelomorph finds itself. What we wish to know is whether or not race *antinorii* possesses modifiers, not present in the absence of mimicry (race *meriones*), which reduce tail length in the female and in particular whether some of these are specific in action to the mimetic forms. The difference in the mean tail length of *meriones* females and the of yellow *antinorii* ones indicates that the two races do differ in modifiers and that the increased variance suggests that there is considerable segregation for them in *antinorii*. The even larger variance in the wild mimetic females of pure *antinorii* and the backcross family is consistent with the view that some of these modifiers are specific to the mimics. Thus in the backcross brood both the longest and shortest tailed homozygous

(*tt*) individual is to be found among the mimetic forms despite the fact that the mean tail length is less than is that of the yellow females. The tailed (*tt*) individuals among the F_2 hybrids are also instructive since in them a greater segregation of modifiers would be expected, if such exist. Moreover, if modifiers specific to mimetic individuals are rarer in the tailless main African races as they might be expected to be (where yellow tailed individuals are absent), there should be an increase of variance and a decrease in the difference in mean tail length between yellow and mimetic females. The data such as they are give no indication of a difference in mean between yellow (9·5 mm.) and mimetic (9·5 mm.) females and do suggest a considerable increase in variance over the backcross (difference in variance between *tt* F_2 and B_1 yellow females $P<0{\cdot}01$).

The genetic evidence on tail length taken as a whole strongly supports the thesis that there is disruptive selection acting which is resulting in the accumulation of modifiers which reduce tail length particularly in the mimics, so that they conform more closely to the models. Evidence for strong disruptive selection in race *antinorii* has already been mentioned by Clarke and Sheppard (1960). They pointed out that the presence of dominance between the yellow form and *hippocoonides* and *cenea* but its absence between yellow and its allopatric forms strongly suggests that disruptive selection has already resulted in the elimination of intermediate heterozygotes which would not possess either the advantageous pattern of the mimics or the male-like female.

Over and above the evidence of the genetics of the tailed condition and of dominance there is no doubt from the results so far obtained (race crosses using *hippocoon*, *hippocoonides*, *cenea*, *trophonius* and *planemoides*) that a gene controlling a mimetic pattern produces a closer resemblance to the model in the gene-complex of a wild population where both mimic and model are found than in one where the model and mimic are absent or than in a hybrid genotype. Furthermore there is some evidence from *cenea* and *niavioides* that when the mimic is relatively uncommon the mimetic resemblance is sometimes less perfect than in a population in which it and the model are common. When the mimic becomes relatively very abundant compared with the model mimicry again begins to break down (race *polytrophus*). This would be expected since, when the mimic is " too common ", birds cannot learn that the pattern indicates inedibility in the model, the advantage of the mimetic pattern vanishes and selection no longer stabilises it.

Since the genes we are considering do not produce perfect mimicry in all the gene-complexes of the species we must conclude that, if Goldschmidt's hypothesis is correct, an allelomorph producing mimicry can only establish itself if by chance it occurs in a very favourable gene-complex. Moreover, we must also conclude that once established, selection is powerful enough to stabilise the gene-complex

so that the good resemblance produced by the gene is maintained. Such an extreme view does not accord with the facts. In *P. dardanus* the mimetic forms are found wherever the models are common so that on Goldschmidt's view a suitable gene-complex must have occurred by chance (at a time when the mutant was available) in all areas where appropriate models are at high frequency notwithstanding that in other areas, anyhow at the present time, such suitable gene-complexes do not occur! Now we know from the work of Brower (1958) that in the laboratory, in circumstances in which birds could examine their prey for two minutes, imperfect resemblances protect to some extent. It is therefore unreasonable to assume that in the wild, when the bird may see the insect for only a few seconds, imperfect mimicry will not confer some advantage. This is in accord with the finding that where the mimic is rare, mimicry is sometimes less perfect. It has already been shown that if we accept Goldschmidt's view we must also assume that selection is powerful enough to stabilise the gene-complex to give good mimicry even if the mimic only accounts for some 10 per cent. of the female population. But such powerful selection would also be strong enough to alter the gene-complex to give a better mimetic resemblance if an imperfect mimic established itself. We may therefore safely reject Goldschmidt's hypothesis and accept that developed by Fisher and Ford.

5. SUMMARY

1. The main polymorphic forms of *P. dardanus* are controlled by a multiple allelomorphic series.

2. The varieties *hippocoon* and *hippocoonides*, which mimic different subspecies of the model *A. niavius*, differ at three and probably many more than three loci.

3. Allelomorphs controlling the varieties *hippocoonides*, *cenea*, *trophonius* and *planemoides* produce a much less perfect resemblance to the models when the gene-complex is modified by hybridisation with a race not possessing the particular mimic.

4. The absence of tails on the hindwings of the main African races is due to a single gene, the condition showing incomplete dominance.

5. In the tailed Abyssinian and Madagascan races the length of the tails is multifactorially controlled. In the monomorphic Madagascan race the variance of tail length is as small in the females as it is in the males of all races. In Abyssinia, the females have a shorter mean tail length than the males and greater variance, these features being particularly marked in the mimetic individuals.

6. The results support the view that the original mimetic resemblance produced by a new mutant is enhanced by the selection of modifiers and when perfected the pattern is stabilised by selection. In Abyssinia there appears to be disruptive selection which is accumulating modifiers reducing female tail length in the mimics. Owing to

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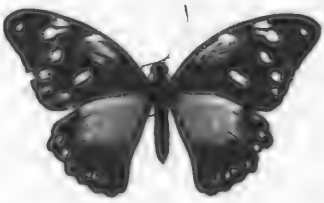

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Plate I

1. *Amauris niavius dominicanus* from East and South Africa.
2. *A. n. niavius* from West Africa.
3. *P. dardanus* form *hippocoonides* mimicking 1.
4. Form *hippocoon* mimicking 2.
5. The ubiquitous *Danaus chrysippus*.
6. *Amauris albimaculata* from Central, East and South Africa.
7. Form *trophonius* mimicking 5.
8. Form *cenea* mimicking 6.

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Plate II

1. Heterozygote from a South African *cenea* × Central African *hippocoon*. It is similar in pattern to the local Central African *cenea*.
2. Heterozygote from a South African *cenea* × West African *hippocoon*. The mimicry in this form is less perfect particularly with respect to the forewing white spots.
3. Heterozygote from a South African *trophonius* × West African *hippocoon*. In this cross the mimicry remains well developed in contrast to 2.
4. to 8. These are examples from an F_2 family from a South African *hippocoonides* stock × a Central African *hippocoon* stock. The insects are chosen to show the extremes of development and the independent assortment of the differences between *hippocoon* and *hippocoonides* (see text).

the segregation of these modifiers there is greater variance of tail length in the polymorphic Abyssinian than in the monomorphic Madagascan race.

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6. REFERENCES

BROWER, J. 1958. Experimental studies of mimicry in some North American butterflies. Part 3. *Danaus gilippus berenice* and *Limenitis archippus floridensis*. *Evolution, 12*, 273-285.

CARPENTER, G. D. H., AND FORD, E. B. 1933. *Mimicry*. Methuen, London.

CLARKE, C. A., AND SHEPPARD, P. M. 1959*a*. The breeding of *Papilio dardanus* Brown in England. Technique and some results. *The Entomologist, 92*, 89-95.

CLARKE, C. A., AND SHEPPARD, P. M. 1959*b*. The genetics of some mimetic forms of *Papilio dardanus* Brown, and *Papilio glaucus* Linn. *J. Genet., 56*, 236-259.

CLARKE, C. A., AND SHEPPARD, P. M. 1960. The evolution of dominance under disruptive selection. *Heredity, 14*, 73-87.

DARWIN, C. 1872. *The Origin of Species*, 6th ed. Murray, London.

FISHER, R. A. 1930. *The Genetical Theory of Natural Selection*. Clarendon Press, Oxford.

FORD, E. B. 1936. The genetics of *Papilio dardanus* Brown (Lep.). *Trans. Roy. Ent. Soc. Lond., 85*, 435-466.

FORD, E. B. 1953. The genetics of polymorphism in the Lepidoptera. *Advance. Genet., 5*, 43-87.

GOLDSCHMIDT, R. B. 1945. Mimetic polymorphism, a controversial chapter of Darwinism. *Quart. Rev. Biol., 20*, 147-164, 205-230.

MATHER, K. 1955. Polymorphism as an outcome of disruptive selection. *Evolution, 9*, 52-61.

PUNNETT, R. C. 1915. *Mimicry in Butterflies*. Cambridge Univ. Press, London.

THODAY, J. M. 1959. Effects of disruptive selection. *Proc. 15th Int. Cong. Zool.*, pp. 127-130.

SUPER-GENES AND MIMICRY

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1. INTRODUCTION

It has been shown (Clarke and Sheppard, 1960*a*) that only two loci control the main differences between the various mimetic and non-mimetic forms of the female of the African butterfly *Papilio dardanus*. As far as colour and pattern are concerned only one locus apparently is responsible, the presence or absence of " tails " on the hindwing being determined by the other. This mode of genetic determination does not necessitate that each pattern arose fully perfected as the result of a single mutation, but only that the various allelomorphs act as a " switch mechanism ", the actual patterns thus switched having evolved gradually as the result of the accumulation of modifiers (see Ford, 1953; Clarke and Sheppard, 1960*b*). Moreover, as we will attempt to show in this paper, it is probable that the switch mechanism itself evolved by a series of small steps and what now appears to be a single locus is really a super-gene.

2. THE PRIMITIVE PATTERN

It is impossible to determine with certainty the primitive colour-pattern of *P. dardanus* (that present just before the first mimic evolved) since this is a matter of pre-history. However, it is possible to make an enlightened guess on the matter. The males of this species are everywhere the same in general pattern although the details vary slightly from race to race. They all have tailed hindwings and a black and yellow pattern, the yellow pigment fluorescing under ultra-violet light. Even the very distinct subspecies or species *humbloti* from Grand Comoro has this type of male although the shape of its wings, the exact distribution of black pigment and the length of the tails are somewhat different. The males of this form also differ from those of the eastern races of *dardanus* in the structure of their genitalia which are like those of the western race. We must therefore either conclude that all the various males have (i) previously diverged and then converged with respect to pattern, (ii) evolved in parallel with one another, or (iii) remained fairly constant, the present pattern being that of the common ancestor of all the races. The third view seems the most likely for several reasons.

1. Yellow fluorescent pigment and the presence of tails are characteristic of a large proportion of the members of the genus *Papilio* suggesting that these were possessed by a common ancestor.

2. *Papilio nobilis*, a distinct species (with several subspecies) from East Africa, considered to be closely related to *P. dardanus* but having a different ecology particularly in the immature stages, also has tails and the same general colour-pattern as *P. dardanus* but with the black areas replaced by brown. Since this species is closely related to *P. dardanus* but ecologically distinct it seems more likely that the common colour-pattern is due to the retention of a common ancestral form rather than to convergence.

3. In other butterflies in which the females are polymorphic or mimetic but the males monomorphic and non-mimetic the latter usually resemble closely related species. Since there are many such examples (*Speyeria diana*, *Argynnis paphia*, *Papilio glaucus*, *P. cynorta*, *P. echerioides*, *Hypolimnas misippus* and *H. bolina* to mention only a few), it seems unlikely that all can be due to convergence. It is more probable that the close resemblance between the males of related species reflects the conservation of an ancestral pattern. The reason for the relative constancy of male pattern may reside in the nature of butterfly courtship. It is known that colour is important in butterfly mating behaviour and Ford (1953) has suggested that this is particularly true in the male and that a novel colour-pattern fails to stimulate the female to copulate. Consequently male colour-pattern is particularly conservative, as demonstrated by the rarity of polymorphism in male butterflies as compared with females.

For the reasons given, it seems likely that the male colour-pattern is older than the races of *P. dardanus*. This does not tell us whether the females also had this pattern since it is possible that the species had become sexually dimorphic before the mimicry had been evolved. In those populations of *P. dardanus* where the mimicry is absent (Grand Comoro, *humbloti*, and Madagascar, *meriones*), there is no marked sexual dimorphism, the females being tailed and having the yellow and black pigments of the male. In the only other race which is well isolated (*antinorii*), all the females have tails and those with a male-like pattern are the commonest form, constituting some 80 per cent. of the female population. There is some gene exchange between all the other continental races, at the present time, and none has females possessing tails or the male-like pattern. One, *polytrophus* from Kenya, does possess, however, a small proportion of insects which have varying amounts of fluorescent yellow pigment (see below).

That the two races without mimics are not sexually dimorphic suggests that the male-like pattern and the presence of tails may be ancestral in the female, particularly in view of the fact that (i) the female pupa has tails in all races, including those in which the female butterfly has not got them, a very strong indication that this character is primitive, and (ii) the female of *P. nobilis* has a very similar appearance to its own male and the non-mimetic yellow males and females of *P. dardanus*. If we assume that the black and yellow tailed form is not primitive we must conclude that the similarity between the females

of races *meriones*, *humbloti* and those of the species *P. nobilis*, as well as that of the yellow (male-like) ones in race *antinorii*, is due to convergence. The geographical disposition makes it more than unlikely that *antinorii* could have been established directly from race *meriones* or vice versa.

One might argue that in the case of these two races convergence in respect of yellow pigment is extremely probable since the presence of yellow pigment is controlled by a single allelomorph. However, it is not only with respect to the presence of tails and yellow pigment that the forms are similar. The details of the black pattern, which are multifactorially controlled, are also much alike in the females. A cross between *meriones* and *antinorii* might distinguish between convergence and common ancestry. If it were found that the same modifiers controlled the distribution of black in both races it would all but rule out convergence since the probability of using the same modifiers to evolve the male-like pattern in both races is negligible. Even in the absence of such data it seems reasonable to proceed on the assumption that *P. dardanus* was originally monomorphic, tailed and with a black and yellow appearance similar to that of the present-day males.

3. THE ORIGIN OF MIMICRY IN ABYSSINIA

The Abyssinian race of *P. dardanus* is, at the present day, well isolated from the other races. It is therefore of some interest to consider how the mimicry arose in this area. We can assume with a fair degree of certainty that the mimicry in Abyssinia is being maintained by selection since appropriate models are present and the tails of the females, particularly those of the mimics, although not absent, are reduced compared with those of males and Madagascan females, as the result of the presence of specific sex-controlled modifiers (Clarke and Sheppard, 1960*c*). Only three (possibly four) mimics are known from this population and, surprising as it may seem, the two mimics tested (*hippocoonides* and *cenea*) are both recessive to the non-mimetic yellow form. Clarke and Sheppard (1960*c*) have obtained evidence for believing that this recessiveness has been evolved owing to the disadvantageous appearance of the original intermediate heterozygote. Now if a mutant producing imperfect mimicry in the heterozygote arose in a monomorphic population one would expect selection for modifiers improving the resemblance to the model. In such circumstances the form is unlikely to become recessive, for to do this the appearance of the heterozygote would have to be disadvantageous which it could not be if it were being selected on its visual appearance, as in mimicry (Sheppard, 1958). Consequently if the original population of females were male-like, the mimics did not arise in Abyssinia by mutation and become established on account of selection for their visual effect, otherwise they would be dominant, not recessive. They

must therefore have been introduced in large numbers by hybridisation. Only thus could the gene-frequency have been raised sufficiently for enough homozygotes to be produced to allow the gene to become established despite the disadvantageous appearance of the heterozygote. The only other reasonable hypothesis to account for the dominance relationships is that the original population was mimetic and that it was the yellow form which arose by mutation and increased in frequency, implying convergence between *antinorii* and *meriones*, an hypothesis which seems slightly the less likely of the two (see above).

The massive and prolonged hybridisation envisaged here could hardly have been all in one direction, so that for supporting data we can look at adjacent races for evidence of hybridisation. Now since the genitalia of *antinorii* males are of the East African type such race mixture as has occurred is likely to be with an eastern race. Moreover, geographical considerations suggest that the most probable race is *polytrophus* from Kenya. An increase in rainfall would allow the forests of the Kenya and Abyssinian highlands to become contiguous by spreading into the dry areas between them, particularly in the vicinity of Lake Rudolf. In fact two areas of mountain-top forest in this intervening region do support, at this moment, populations of *P. dardanus* (of the eastern race) in which a very remarkable mimic *ochracea* has evolved, copying the local common *Amauris echeria septentrionalis*.

In *polytrophus* there are yellow females, proto-*hippocoonides*, which are identical to our *antinorii* yellow/*hippocoonides* heterozygotes (H^{y}*h*) of the second and subsequent backcross generations to Central African stock. There seems little doubt that proto-*hippocoonides* which is found nowhere else (except as a rare aberration in neighbouring races) is a yellow/*hippocoonides* heterozygote and that its differences (the shade of the yellow and the distribution of black pigment) from the yellow Abyssinian females are due to the *polytrophus* gene-complex. If this interpretation of the genetics of proto-*hippocoonides* is correct it militates against the hypothesis of convergence, since there is no indication of this occurring in the *polytrophus* race, and helps to support that of hybridisation by accounting for the presence of yellow females in Kenya but nowhere else in continental Africa except Abyssinia. A polymorphism, however maintained, is likely to result in the heterozygotes becoming advantageous to both homozygotes (Ford, 1940; Sheppard, 1953*a* and see below). Consequently there is no difficulty in accounting for the persistence of the allelomorphs for yellow in Kenya where the effectiveness of the mimicry, and therefore counter selection against intermediate phenotypes (such as proto-*hippocoonides*), is much reduced owing to the scarcity of models (see Sheppard, 1960). Had the models been more abundant one might have expected proto-*hippocoonides* to have been eliminated.

There are other features of the *polytrophus* race which support the hybridisation hypothesis. (i) Not only are the forms in this race more

variable than in any other, as might be anticipated after hybridisation in the absence of strong selection, but the variability is of the type to be expected. Thus there is a tendency for intermediates between otherwise distinct forms to appear, suggestive of a breakdown of dominance similar to that produced in our race-crosses. (ii) Even more convincing is the decided tendency in *polytrophus* for the black bar in area 2 (see fig. 2 in Clarke and Sheppard, 1960*c*) to be reduced or missing, and area 3 to be increased in size, a feature common to our *antinorii* hybrids (Clarke and Sheppard, 1960*b* and *c*). This similarity between forms of race *polytrophus* and the *antinorii* hybrids with Central African stock is particularly marked in bright- and pale-*poultoni*. (iii) In race *polytrophus* alone are found, on rare occasions, females with short tails. Such females are identical in appearance with tailed heterozygotes (*Tt*) in our backcross families of the Abyssinian and Madagascan tailed races (*tt*) to Central African (*TT*) stock. The presence of these females in *polytrophus* suggests that the allelomorph *t* is still present, albeit at a very low frequency. (iv) The males of the race have genitalia of the eastern races but reduced black markings which are so characteristic of *antinorii* and not of the other eastern forms.

The chief difficulty with the hypothesis would seem to be in explaining the absence of the tailless condition in Abyssinia today if the females of the Kenya populations were already tailless at the time of hybridisation. On the other hand one might maintain that the females were tailed at that time. This is altogether unsatisfactory since we would also have to assume that despite the weak selection for mimicry, as indicated by the presence of proto-*hippocoonides* and other imperfect mimics, it has been sufficiently strong to establish the allelomorph *T* at high frequency even though its advantage lay only in the improved mimicry it afforded. However, the presence of the tailed condition in all *antinorii* females is consistent with hybridisation with a tailless Kenya race. The locus concerned is not linked with that controlling colour-pattern and consequently the two conditions will tend to be distributed at random in a population polymorphic for both. Now, even if there is strong selection for the absence of tails in mimetic Abyssinian females, a mild disadvantage of this condition in the yellow females would ensure the elimination of the allelomorph (particularly a semi-dominant one) since less than 20 per cent. of the females are mimetic. In Kenya the yellow females are rare (about 6 per cent., Ford, 1936) and in consequence the reverse process would occur, although more slowly, owing to the mildness of the selection for mimicry and the semi-recessiveness of the tailed character. This would account for the continued presence of the gene *t* at low frequency in race *polytrophus*. That there is, in fact, selection against tails in the mimics and for them in the yellow females is supported by the fact that the yellow Abyssinian females have longer tails than do the mimics.

4. THE ORIGINAL MIMIC

It is as difficult to decide the pattern of the first mimic as it is to determine the primitive female colour-pattern. We have, however, some theoretical points to help us. It was pointed out by Sheppard (1958) that a gene which is selected because of its advantageous effect on the appearance of an animal is likely to become dominant if it and its allelomorph are retained in a population. Moreover, such an allelomorph initially has a better chance of establishing itself if it is not entirely recessive in its effect. For similar reasons a second mutant is likely to become dominant to the first. Consequently the first mimic to appear is likely to be recessive to all others but semi-dominant to the original form when in a gene-complex which has never experienced the mutant. It is of course possible that the second mutant arose before the first was very common, in which case the latter might become recessive to it. We can therefore only say that the bottom recessive mimic is likely to be the oldest or at least established while the non-mimetic form was still the commonest variety.

Now *hippocoonides* (and its modifications *hippocoon* in West Africa, and *niavioides* in Abyssinia), besides being the most widely distributed and frequently the commonest mimic, is also recessive to all other sympatric forms. It is therefore probable that it was the first of the extant mimics to evolve. Such a view becomes even more plausible when we consider that a widespread and common model such as *A. niavius*, whose presence exerts a powerful selective effect as judged by the high frequency of *hippocoonides* in many areas, has a better chance of evoking mimicry than one with a restricted range and therefore a selective influence on only a restricted proportion of the potentially mimetic species. The gene controlling *hippocoonides* also has the necessary attribute of semi-dominance to the presumed primitive yellow females of the monomorphic Madagascan race. The heterozygote in the F_1 hybrid with Central African stock produces a good mimetic resemblance as far as the black pattern is concerned and is inferior to *hippocoonides* itself chiefly in the ground colour of the pale areas which is yellow (lighter than in *meriones* females), not white. Such a heterozygote might well possess some mimetic advantage where the form is rare and the model abundant.

Trimen (1870), when the genetics of *P. dardanus* was quite unknown, suggested, from a consideration of the pattern, that mimicry in this species had been based on an extension of the black costal margin of the forewing. This black bar extends from the base along the costa ending near the extremity of the cell and has a tendency to curve backwards distally. An extension of this backward curve to the outer margin of the wing near the inner angle would divide the pale areas in a way characteristic of nearly all the mimetic patterns. Now the allelomorph controlling *hippocoonides* does just this in the heterozygote with the yellow allelomorph in the gene-complex of the Central

African stock and Madagascan hybrids of the F_1 and first backcross generations to the South African race. Thus both the genetic and morphological data are in agreement in singling out *hippocoonides* or some modification of it as the original mimic.

5. THE SUPER-GENE

Both Mather (1955) and Sheppard (1953*b*) have pointed out that once a polymorphism is established there is likely to be a gradual incorporation of new mutants into the "switch mechanism" to produce a super-gene. To illustrate this process one can use the characters presence or absence of tails in mimetic species belonging to the genus *Papilio*. All the available models for *P. dardanus* are tailless since the distasteful Aristolochian swallowtails, which can serve as tailed models in other areas, are not found on the African continent. Consequently a mutant reducing or eliminating the tails will be at an advantage visually whenever most of the females are mimetic. However, as we have argued above, where the mimics are rare and the yellow females common such a character will be, on average, at a disadvantage since, although at a premium in the mimics, it is at a disadvantage in the commoner male-like (yellow) females. There will be, therefore, no balanced polymorphism, the tails being either present or absent depending on the frequency of the mimics and ultimately of the models. Had tailed models been abundant in Africa it is probable that the allelomorph *T* for the absence of tails would never have established itself. In the South American swallowtail *Papilio lysithous* there are three mimics *lysithous*, *pomponius* and *rurik* present in both sexes and mimicking the Aristolochian swallowtails *Atrophaneura nephalion*, *A. perrhebus* and *A. chamissonia* respectively, the first two of these models being tailed and the last tailless. However, all the mimics have tails and in this follow the character of the majority of models. It seems fairly certain that a mutant eliminating the tails, had it occurred in the same chromosome as the allelomorph giving *rurik*, would have put insects possessing it at an advantage and a polymorphism with respect to the presence or absence of tails would have ensued. Moreover, since any crossover between the loci concerned, in the double heterozygote, would result in a disadvantageous arrangement of the pairs of allelomorphs there would be selection against it and the loci controlling colour-pattern and tails would become incorporated into a super-gene (Kimura, 1956; Mather, 1955; Sheppard, 1953*b*).

Just such a super-gene seems to be present in *Papilio memnon*. In this species there are two non-mimetic tailless forms of which the genetics are known, *laomedon* which is like the male, and *isarcha*, the latter being due to a sex-controlled gene dominant in effect. A second unlinked dominant converts *isarcha*, but not *laomedon*, into *achates*, a tailed mimic of the Aristolochian tailed swallowtail *Atrophaneura coon*. Other than the presence of two closely linked loci, controlling pattern and

tails respectively, there seem to be only two possible explanations for the genetic situation in *P. memnon*: (i) the rather unlikely one that the mutant by chance controls both colour-pattern and wing shape or (ii) that there has been strong selection for sex-controlled modifiers which interact with *achates* but not *laomedon* or *isarcha* to produce tails, in the same way that modifiers reducing tail length in the mimics of *P. dardanus*, race *antinorii*, seem to be being selected at the present time. Whichever of these explanations is correct the principle seems clear that mutants which are advantageous in the presence of one form but disadvantageous in that of its alternative are not likely to establish a polymorphism unless the two responsible loci are or become closely linked (Kimura, 1956; Sheppard, 1955). Thus once a polymorphism is established as the result of disruptive selection one must expect the gradual accumulation of other mutants on the controlling chromosome and the formation of a super-gene as Mather (1955) predicted.

The same principle should hold equally for the different elements of a mimetic colour-pattern and we found on examining the various forms of *P. dardanus* that they are made up of different combinations of a few simple elements such as the distribution of black on the forewings and on the hindwings as well as variations in the ground colour of the pale areas of both wings. It therefore seems possible that the apparent multiple allelomorphic series controlling the colour-pattern in all forms investigated is really a super-gene in which various combinations of linked allelomorphs control each pattern. There is no direct evidence for this hypothesis from the breeding data since no crossover has been detected. If selection for close linkage has been prolonged, crossing-over is likely to be almost as rare as mutation. There is some indirect evidence for the presence of a super-gene. The form *niobe* is controlled by a single genetic unit and the form is dominant to *hippocoonides*. The same mimic is also produced by the heterozygote between the factors for *trophonius* and *planemoides* both of which are sympatric with *niobe*. Now a crossover in this heterozygote could produce a combination which would give *niobe* inherited as a single unit (the other crossover class being *hippocoonides*). Consequently a possible mechanism for producing a mimic by crossing-over exists and if the gene-complex had already been adjusted to the heterozygote the mimicry would be good from its first occurrence. The other evidence for a super-gene comes from race *polytrophus*, in which the models are rare and the mimicry poor. Now it is in just this race, and this race alone, that mimics with yellow fluorescent pigment are found and it is here also that the allelomorph producing the yellow of *antinorii* is also found (proto-*hippocoonides*). These mimics with this yellow pigment are not heterozygous for proto-*hippocoonides*. It therefore seems not unreasonable to suppose that these imperfect mimics in which the white areas are replaced by yellow male pigment, result from crossing over within the super-gene between parts controlling the mimetic pattern and that determining the presence of yellow, and

that these products have not been eliminated because of the scarcity of the models. It is perhaps of importance to note that one of these imperfect mimics, proto-*trophonius* (apparently controlled by an allelomorph of *planemoides*, Clarke and Sheppard, 1960*a*) is an exact replica in colour-pattern of a heterozygote between *trophonius* and the yellow Madagascan form which behaves as an allelomorph of it.

The hypothesis put forward by Mather (1955) that disruptive selection could lead to the establishment of a polymorphism controlled by a super-gene is in full agreement with the data from *P. dardanus* as well as with the artificial selection experiments of Thoday (1959). Moreover, the data further suggest that when there are a large number of optimum phenotypes which differ by combinations of a limited number of characters there are likely to evolve a number of allelomorphs of the super-gene as the result of selection for some but not all of the crossover classes within the super-gene. Thus it is not impossible that *hippocoonides* itself was not the first mimetic pattern but that the first element was one giving an extension of the black costal border of the female and that later a second mutant which replaced the yellow pigment by white was incorporated. Subsequently other mimetic patterns could have been evolved as new mutants giving non-fluorescent yellow or orange-brown pigment arose and still others modified the distribution of black pigment.

The presence of a super-gene not only explains the apparent participation of only one locus in the control of the colour-patterns but also reduces the difficulty felt by many people of imagining the evolution of a gene-complex which is adjusted to many allelomorphs in one area. If the pattern is controlled by combinations of relatively few linked mutants the gene-complex only has to be adjusted to each factor individually for them to produce the optimum effect in all their many combinations. Such an adjustment would explain why the black bar in area 2 of the *antinorii* F_1 hybrids is absent or much reduced, not only in *cenea*/yellow and *hippocoonides*/yellow heterozygotes in which the dominance of yellow has been evolved in *antinorii* (Clarke and Sheppard, 1960*c*), but also in heterozygotes using the allopatric forms *trophonius*, *natalica*, bright-*poultoni*, pale-*poultoni* and *leighi*.

A marked feature of mimicry in *P. dardanus*, the persistence of many different non-mimetic forms which are unlike the males, is also explained by the super-gene hypothesis. The various linked allelomorphs are certain to have other effects than those on colour, some of which will be advantageous and others disadvantageous (see Caspari, 1950). Now the advantageous effects will become dominant and the disadvantageous ones recessive; we already have evidence that they do so in the case of colour-pattern. Consequently on average the heterozygotes will come to be at an advantage to the homozygotes (Sheppard, 1953*a*) and the polymorphism is likely to persist even in the absence of models. Moreover, crossovers will sometimes incorporate these advantages of the heterozygote sufficiently markedly for them to persist

even if the resulting pattern is not mimetic (for example *leighi* or *natalica*). The chance of persistence will be particularly great where the selection for mimicry is most relaxed, as in *polytrophus*. Now it is in just this race that the imperfect mimics and non-mimetic forms are particularly well represented.

6. SUMMARY

None of the evidence is conclusive on its own but there seems little doubt, when all of it is considered together, that:

1. The pre-mimetic pattern of the female was similar to that of the male of the present time.

2. The presence of mimics in race *antinorii* (from Abyssinia), although maintained by selection, was originally due to hybridisation with race *polytrophus* from Kenya and not to mutation within the race.

3. The original mimetic pattern resulted from a mutant causing an extension of the black pigment of the female's costal border towards the inner angle of the forewing.

4. Other loci have become incorporated in the chromosome pair carrying the original mutant and have formed a super-gene which now exists in various forms as the result of recombination and selection.

5. The persistence of the polymorphism over a long period has resulted in the evolution of a selective superiority of some heterozygotes.

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7. REFERENCES

CASPARI, E. 1950. On the selective value of alleles *Rt* and *rt* in *Ephestia kühniella*. *Amer. Nat.*, *84*, 367-380.

CLARKE, C. A., AND SHEPPARD, P. M. 1960*a*. The genetics of *Papilio dardanus* Brown. Pt. I, II and III *Genetics* (in press).

CLARKE, C. A., AND SHEPPARD, P. M. 1960*b*. Evolution of mimicry in the butterfly *Papilio dardanus* Brown. *Heredity 14*, 163-173.

CLARKE, C. A., AND SHEPPARD, P. M. 1960*c*. The evolution of dominance under disruptive selection. *Heredity 14*, 73-87.

FORD, E. B. 1936. The genetics of *Papilio dardanus* Brown (Lep.). *Trans. Roy. Ent. Soc. Lond.*, *85*, 435-466.

FORD, E. B. 1940. Polymorphism and taxonomy. In *The New Systematics*, ed. Huxley, Oxford Univ. Press, London.

FORD, E. B. 1953. The genetics of polymorphism in the Lepidoptera. *Advanc. Genet.*, *5*, 43-87.

KIMURA, M. 1956. A model of a genetic system which leads to closer linkage by natural selection. *Evolution*, *10*, 278-287.

MATHER, K. 1955. Polymorphism as an outcome of disruptive selection. *Evolution*, *9*, 52-61.

SHEPPARD, P. M. 1953*a*. Polymorphism and population studies. *Evolution Soc. Exp. Biol. Symp.*, *7*, 274-289.

SHEPPARD, P. M. 1953*b*. Polymorphism, linkage and the blood groups. *Amer. Nat.*, *87*, 238-294.

SHEPPARD, P. M. 1955. Genetic variability and polymorphism, a synthesis. *Cold Spring Harbor. Symp. Quant. Biol.*, *20*, 271-275.

SHEPPARD, P. M. 1958. *Natural Selection and Heredity*. Hutchinson, London.

SHEPPARD, P. M. 1960. The evolution of mimicry ; a problem in ecology and genetics. *Cold Spring Harbor. Symp. Quant. Biol.*, *24*, 131-140.

THODAY, J. M. 1959. Effects of disruptive selection. *Proc. 15th Int. Cong. Zool.*, pp. 127-130.

TRIMEN, R. 1870. On some remarkable mimetic analogies among African butterflies. *Trans. Linn. Soc. Lond.*, *26*, 497-522.

MIXED SIB AND RANDOM MATING WHEN HOMOZYGOTES ARE AT A DISADVANTAGE

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1. INTRODUCTION

An investigation of several inbreeding systems when homozygotes are at a selective disadvantage compared with heterozygotes was conducted by Hayman and Mather (1953). Hayman (1953) also considered the system of mixed selfing and random mating which occurs with plants which have imperfect selfing mechanisms. The present investigation is of a further mixed system, that of sib and random mating. Since sib mating is the most intensive inbreeding system possible with animals we are performing for animals what Hayman (1953) did for plants.

A further important mixed system is that of parent-offspring and random mating which corresponds fairly closely to the backcrossing system practised in cattle breeding. This would be even more difficult mathematically than the present investigation but Hayman and Mather (1953, figs. 3 and 4) showed that pure parent-offspring mating achieved a similar level of inbreeding to pure sib mating whatever the selective disadvantage of the homozygotes. We may, therefore, expect our present conclusions to be equally valid for mixed parent-offspring and random mating.

We also compare the relative inbreeding effectiveness of selfing and sib mating when some random mating is permitted. In the situation discussed by Hayman and Mather (1953) we can make this comparison with the relative survival rates of homozygotes that produce equal levels of heterozygosity under the two mating systems and we find that selfing is about 60 per cent. more effective as an inbreeding system than sib mating for homozygote survival rates down to 20 per cent., but that as the survival rate is lowered further this difference in inbreeding effectiveness gradually disappears. We will see that with a proportion of random mating the relative inbreeding effectiveness of selfing and sib mating is considerably altered.

2. GENERAL EQUATIONS

One gene with two alleles, a and b, is considered, the population being assumed large enough for random variations in frequency to be neglected. The population can be classified into six distinct mating types

appropriate to sib mating. Let the relative frequencies of the types $aa \times aa$, $bb \times bb$, $aa \times bb$, $ab \times ab$, $aa \times ab$, and $bb \times ab$ be λ, μ, α, β, γ, δ respectively, $(\lambda+\mu+\alpha+\beta+\gamma+\delta = 1)$, and let $x, y, 1$ be the relative viabilities of the genotypes *aa*, *bb* and *ab*.

Starting from a population $(\lambda_0, \mu_0, \alpha_0, \beta_0, \gamma_0, \delta_0)$ the genotypic array in the next generation is

$$l_0 x\ aa + m_0 y\ bb + n_0\ ab$$

where

$$l_0 = \lambda_0 + \tfrac{1}{4}\beta_0 + \tfrac{1}{2}\gamma_0 \quad m_0 = \mu_0 + \tfrac{1}{4}\beta_0 + \tfrac{1}{2}\delta_0 \quad n_0 = \alpha_0 + \tfrac{1}{2}\beta_0 + \tfrac{1}{2}\gamma_0 + \tfrac{1}{2}\delta_0$$

and hence

$$l_0 + m_0 + n_0 = 1.$$

If $l_0 x + m_0 y + n_0 = k_0$ then the frequencies of the genotypes *aa*, *bb*, *ab* in the next generation are p_1, q_1, r_1 respectively $(p+q+r = 1)$ where

$$k_0 p_1 = l_0 x \qquad k_0 q_1 = m_0 y \qquad k_0 r_1 = n_0.$$

Hence the genotypic array may be written

$$k_0(p_1 aa + q_1 bb + r_1 ab).$$

Thus for random mating with selection the proportions of the mating types in the next generation are

$$k_0^2(p_1^2,\ q_1^2,\ 2p_1q_1,\ r_1^2,\ 2r_1p_1,\ 2q_1r_1).$$

For sib mating the changes in frequency between the generations are specified by the generation matrix **A** (Hayman and Mather, 1953, section 4.2). **A** is the matrix.

Offspring	Parents					
	$aa \times aa$	$bb \times bb$	$aa \times bb$	$ab \times ab$	$aa \times ab$	$bb \times ab$
$aa \times aa$	x	.	.	$\frac{1}{4}x^2$	$\frac{1}{2}x^2$	.
$bb \times bb$	.	y	.	$\frac{1}{4}y^2$	.	$\frac{1}{2}y^2$
$aa \times bb$	.	.	.	$\frac{1}{2}xy$	.	.
$ab \times ab$	.	.	1	1	$\frac{1}{2}$	$\frac{1}{2}$
$aa \times ab$	.	.	.	x	x	.
$bb \times ab$	.	.	.	y	.	y
Column divisors	1	1	1	$x+y+2$	$x+1$	$y+1$

Thus $\boldsymbol{\lambda}_1 \propto \mathbf{A}\boldsymbol{\lambda}_0$ where $\boldsymbol{\lambda}$ is the 6×1 column vector of the mating type frequencies.

Since each column of the generation matrix sums to the proportion of offspring from the corresponding mating type, the constant of proportionality is $1/k_0(p_1+q_1+r_1) = 1/k_0$.

Let s and t ($s+t=1$) be the frequencies of sib and random mating respectively in the mixed system. The equations governing the frequencies in the next generation are now

$$\left.\begin{aligned}
k_0(s+k_0t)\lambda_1 &= s\left(x\lambda_0+\frac{x^2}{4(x+y+2)}\beta_0+\frac{x^2}{2(x+1)}\gamma_0\right) && +tk_0^2p_1^2\\
k_0(s+k_0t)\mu_1 &= s\left(y\mu_0+\frac{y^2}{4(x+y+2)}\beta_0+\frac{y^2}{2(y+1)}\delta_0\right) && +tk_0^2q_1^2\\
k_0(s+k_0t)\alpha_1 &= s\frac{xy}{2(x+y+2)}\beta_0 && +2tk_0^2p_1q_1\\
k_0(s+k_0t)\beta_1 &= s\left(\alpha_0+\frac{1}{x+y+2}\beta_0+\frac{1}{2(x+1)}\gamma_0+\frac{1}{2(y+1)}\delta_0\right) && +tk_0^2r_1^2\\
k_0(s+k_0t)\gamma_1 &= s\left(\frac{x}{x+y+2}\beta_0+\frac{x}{x+1}\gamma_0\right) && +2tk_0^2r_1p_1\\
k_0(s+k_0t)\delta_1 &= s\left(\frac{y}{x+y+2}\beta_0+\frac{y}{y+1}\delta_0\right) && +2tk_0^2q_1r_1
\end{aligned}\right\}\text{(i)}$$

3. EQUILIBRIUM

The population is in equilibrium if $\lambda_1=\lambda_0$. Let the equilibrium population λ, be $(\lambda, \mu, \alpha, \beta, \gamma, \delta)$ with genotypic frequencies (p, q, r), the two frequency distributions being related by the equations

$$p=\lambda+\tfrac{1}{2}\alpha+\tfrac{1}{2}\gamma \qquad q=\mu+\tfrac{1}{2}\alpha+\tfrac{1}{2}\delta \qquad r=\beta+\tfrac{1}{2}\gamma+\tfrac{1}{2}\delta$$

(which also hold in each generation for any population). Hence, $p-q=(\lambda-\mu)+\tfrac{1}{2}(\gamma-\delta)=l-m$ where, as before, $l=\lambda+\tfrac{1}{4}\beta+\tfrac{1}{2}\gamma$, $m=\mu+\tfrac{1}{4}\beta+\tfrac{1}{2}\delta$, $n=\alpha+\tfrac{1}{2}\beta+\tfrac{1}{2}\gamma+\tfrac{1}{2}\delta$.

Since the population is in equilibrium $kp=lx$ and $kq=my$; $k=lx+my+n$. Also $l+m+n=1$.

Eliminating l, m, n from these equations gives

$$\left.\begin{aligned}
y(k-x)p-x(k-y)q &= 0\\
kyp+kxq+kxyr &= xy\\
p+q+r &= 1
\end{aligned}\right\}\text{(ii)}$$

Therefore

$$\frac{p}{x(1-k)(k-y)}=\frac{q}{y(1-k)(k-x)}=\frac{r}{2(k-x)(k-y)}$$

$$=\frac{1}{k[(k-x)(1-y)+(k-y)(1-x)]} \qquad \text{(iii)}$$

Substituting these results in equations (i) gives six linear equations in $(\lambda, \mu, \alpha, \beta, \gamma, \delta)$, their solution being functions of k. Then using the

equation $\lambda+\mu+\alpha+\beta+\gamma+\delta = 1$ an equation is obtained for k in terms of the parameters x, y, s and t. Thus k is known and hence so is the equilibrium distribution. The elimination of λ, μ, α, β, γ, δ is easily performed by matrix methods. Together with $\lambda+\mu+\alpha+\beta+\gamma+\delta = 1$, equations (i) for equilibrium may be written

$$k(s+tk)\begin{pmatrix}\boldsymbol{\lambda}\\ 1\end{pmatrix} = \begin{pmatrix}s\mathbf{A} & k^2t\mathbf{z}\\ k(s+tk)\mathbf{u} & 0\end{pmatrix}\begin{pmatrix}\boldsymbol{\lambda}\\ 1\end{pmatrix}$$

$$\text{or } k(s+tk)\begin{pmatrix}\boldsymbol{\lambda}\\ 1\end{pmatrix} = B\begin{pmatrix}\boldsymbol{\lambda}\\ 1\end{pmatrix} \qquad \text{(iv)}$$

with $\mathbf{u} = (1, 1, 1, 1, 1, 1)$ and $\mathbf{z} = \begin{bmatrix}p^2\\ q^2\\ 2pq\\ r^2\\ 2rp\\ 2qr\end{bmatrix}$ $\quad p = p(k)$, $q = q(k)$, $r = r(k)$

The equation determining k may be written

$$|\,\mathbf{B}-k(s+tk)\,\mathbf{I}\,| = 0.$$

$\mathbf{I}$ is the 7×7 identity matrix. When $t = 0$, the equation reduces to $|\,\mathbf{A}-k\mathbf{I}\,| = 0$, the usual characteristic equation for pure sib mating (Hayman and Mather, 1953, 4.2).

With the appropriate value of k, solving the equations (iv) yields the equilibrium frequencies $\boldsymbol{\lambda}$.

The expansion of the determinant is

$$(k-x)(k-y)f(k, x, y, s) = 0,$$

$f(k, x, y, s)$ being an eighth degree polynomial in k.

If $k = x$, $\boldsymbol{\lambda} = (1, 0, 0, 0, 0, 0)$ and the genotype distribution is $(1, 0, 0)$. Thus the population becomes homozygous *aa*. Similarly $k = y$ corresponds to a homozygous *bb* population. The roots of $f(k, x, y, s) = 0$ give rise to populations $\boldsymbol{\lambda}$ containing both heterozygotes and homozygotes, only one of which, however, is a real population.

$\boldsymbol{\lambda}$ defines a real, stable population equilibrium if it corresponds to the maximum root of the determinantal equation. The maximum root of $f(k, x, y, s) = 0$ lies between the least of x and y, and unity and defines a real population with genotypic frequencies given by equations (iii). This is the stable equilibrium only if $k > x, y$; otherwise one of the homozygous states is the stable solution.

Fig. 1 shows the type of equilibrium population for various values of x, y and s. The areas marked A and B correspond to the homozygous states *aa* and *bb* respectively; in C the population, though containing heterozygotes, has more homozygotes than a population

mating purely at random without selection; in D it contains more heterozygotes; on the boundary between C and D the effects of inbreeding and selection exactly counter each other.

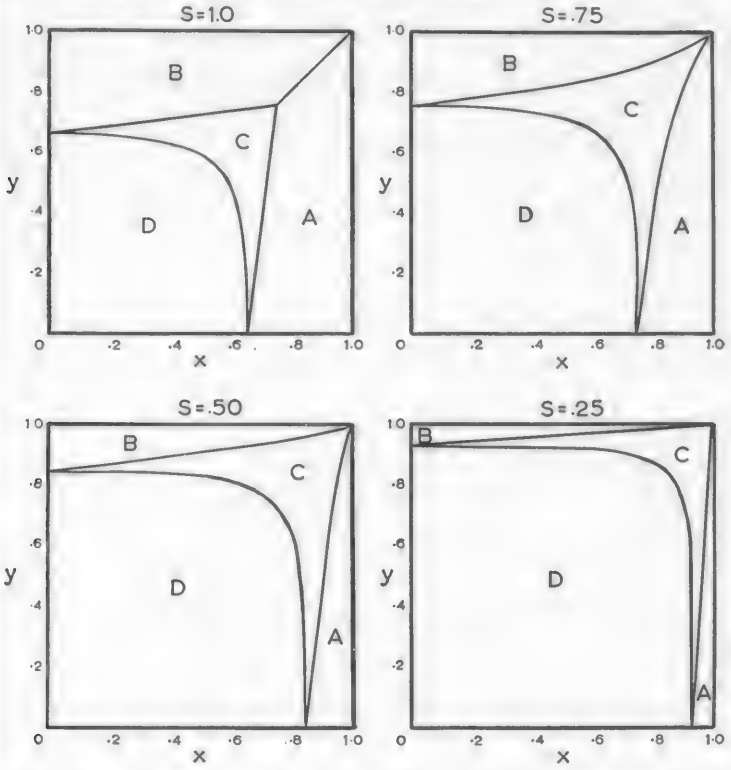

FIG. 1.—Phase diagram showing types of equilibrium with various proportions of sib mating (s) and random mating ($t = 1-s$). x = survival rate of one homozygote (aa) relative to that of the heterozygote (ab). y = relative survival rate of the other homozygote (bb).

When the point (x, y) is in A, the population becomes homozygous aa, in B, homozygous bb. In C, the population becomes less heterozygous than a population mating at random without selection, and in D, more heterozygous.

The inner boundaries of A and B are where two of the three values of k determining the types of equilibrium coincide. Thus their equations are

$$f(x, x, y, s) = 0 \quad \text{and} \quad f(y, x, y, s) = 0.$$

On the boundary between C and D Hardy's law holds, *i.e.* $4pq = r^2$. Thus using this equation with equations (iii), the boundary is

$$f\left(\frac{x+y-2xy}{1-xy}, x, y, s\right) = 0.$$

Unless one allele is initially absent, the equilibrium frequencies are independent of the initial state, being determined by x, y and s only.

4. EQUAL VIABILITIES

When $x = y$ some simplification in the equilibrium distribution is obtained. The first of equations (ii) now becomes

$$(k-x)(p-q) = 0.$$

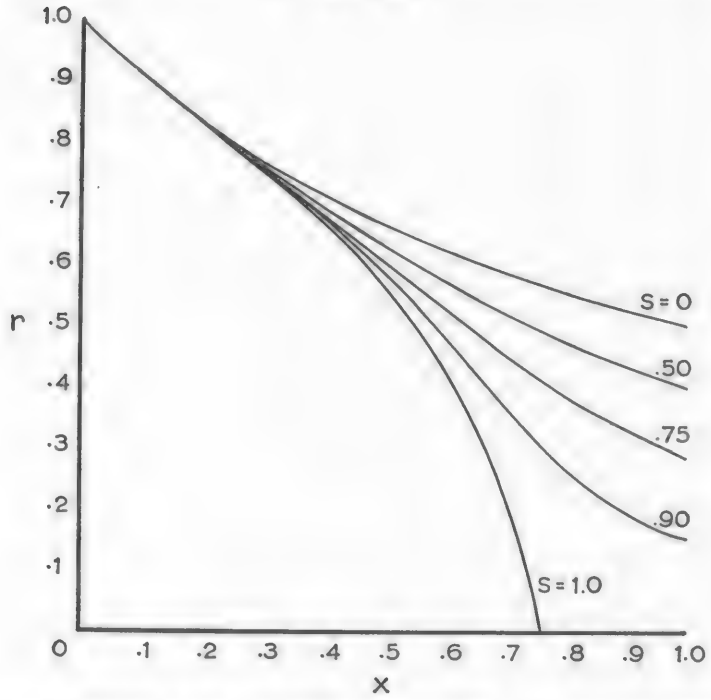

FIG. 2.—Equilibrium with equal viabilities of the homozygotes. r = proportion of heterozygotes in the population. x = survival rate of both homozygotes relative to the heterozygote. s = proportion of sib mating.

If $k = x$ the equilibrium is homozygous with respect to *aa* or *bb*, and these two distributions are unstable, attained only if the initial population has one allele missing. Thus $p = q$ and hence $\lambda = \mu$, and $\gamma = \delta$. Thus in equilibrium the gene frequencies are equal and

$$p = q = \frac{1-k}{2k(1-x)}, \qquad r = \frac{k-x}{k(1-x)} \tag{v}$$

The mating type pairs $aa \times aa$, $bb \times bb$; and $aa \times ab$, $bb \times ab$ may be grouped together, and the matrix **B** is

	$aa \times aa$	$aa \times bb$	$ab \times ab$	$aa \times ab$	$tk^2\mathbf{z}$
$aa \times aa$ . .	sx	.	$\frac{sx^2}{4(x+1)}$	$\frac{sx^2}{2(x+1)}$	$2tk^2p^2$
$aa \times bb$ . .	.	.	$\frac{sx^2}{4(x+1)}$	.	$2tk^2p^2$
$ab \times ab$ . .	.	s	$\frac{s}{2(x+1)}$	$\frac{s}{2(x+1)}$	tk^2r^2
$aa \times ab$ . .	.	.	$\frac{sx}{x+1}$	$\frac{sx}{x+1}$	$4tk^2rp$
$k(s+kt)\mathbf{u}$ . .	$k(s+kt)$	$k(s+kt)$	$k(s+kt)$	$k(s+kt)$	.

The determinantal equation for k is

$$4t^2(1+x)^2k^6+2t(1+x)^2\{4s-(1+x)t\}k^5+2s\{2(1+x)s-(2+6x+3x^2)t\}k^4$$
$$+s(1+x)\{-2(1+4x+2x^2)s+x(2+3x)t\}k^3+x(1+x)(2+3x)s^2k^2$$
$$+sx^3\{(2+x)s+(1+x)t\}k-s^2x^4=0 \qquad \text{(vi)}$$

This equation is also obtained by letting $x=y$ in the general equation $f(k, x, y, s)=0$. The maximum root lies between x and $\frac{1}{2}(1+x)$, and with this value the genotype frequencies are

$$(\tfrac{1}{2}(1-r), \tfrac{1}{2}(1-r), r) \text{ with } r=(k-x)/k(1-x).$$

Graphs of r against x for various s are shown in fig. 2.

5. COMPARISON WITH SELFING

Selfing is the more powerful inbreeding system and a higher proportion of random mating is needed to obtain a given frequency of heterozygotes than in a mixed sib and random mating scheme with the same value, x for the viability of the homozygotes. Fig. 3 is a graph of s_1, the proportion of selfing, against s_2, the proportion of sib mating which gives the same frequency r of heterozygotes in the equilibrium population, for different values of x. Hayman (1953) gives the equation determining r under mixed selfing and random mating, with equal homozygote viabilities as $rx(1+s_1-s_1r)=(1-r)(1-s_1+s_1r)$.

Eliminating r and k between this and equations (v) and (vi) gives the relationship between s_1, s_2 and x.

The ratio $(s_2-s_1)/s_2$ gives a measure of the relative inbreeding effectiveness of the two systems and this in turn can be measured by

the closeness of the (s_1, s_2) curve to the dotted line $s_1 = s_2$. For low homozygote survival rates the curve closely approximates to a straight line whose gradient, s_1/s_2 is constant. For 50 per cent. survival, selfing appears to be about 50 per cent. more effective; for 30 per cent. survival the figure is 80 per cent.; selfing is 90 per cent. more effective

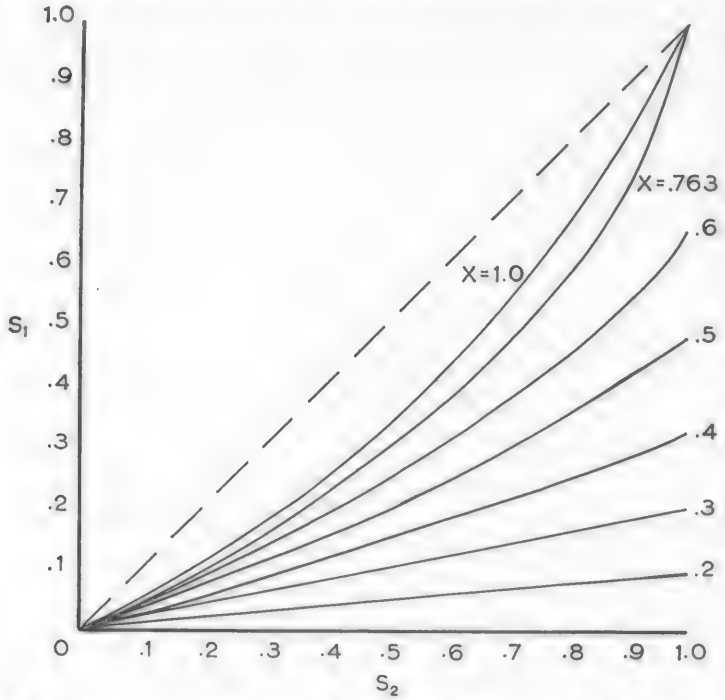

FIG. 3.—Comparison of a mixed sib and random mating system with a mixed selfing and random mating system. s_1 = proportion of selfing. s_2 = proportion of sib mating required to produce the same equilibrium distribution as the mixed selfing system. x = relative survival rate of both homozygotes in either mating system.

Along the broken line, $s_1 = s_2$. The deviation of the (s_1, s_2) curve from this line is a measure of the difference between the inbreeding effectivenesses of the two systems. For small x the curve approximates to a straight line whose gradient measures the effectiveness of the mixed sib mating system relative to that of the mixed selfing system.

For $x > 0{\cdot}763$ the curves meet the line $s_1 = s_2$ at the point (1, 1). All other curves deviate entirely from this line.

for 20 per cent. survival and is almost 100 per cent. more effective for less than 10 per cent. survival of the homozygotes.

5. NO SELECTION OPERATING

The method used above breaks down when $x = y = 1$, but in this case the approach to equilibrium can be described. The equations

linking the mating type frequencies between the nth and $(n+1)$th generations are

$$\begin{aligned}
\lambda_{n+1} &= s(\lambda_n+\tfrac{1}{16}\beta_n+\tfrac{1}{4}\gamma_n) &&+tp_{n+1}^2\\
\mu_{n+1} &= s(\mu_n+\tfrac{1}{16}\beta_n+\tfrac{1}{4}\delta_n) &&+tq_{n+1}^2\\
\alpha_{n+1} &= s(\tfrac{1}{8}\beta_n) &&+2tp_{n+1}q_{n+1}\\
\beta_{n+1} &= s(\alpha_n+\tfrac{1}{4}\beta_n+\tfrac{1}{4}\gamma_n+\tfrac{1}{4}\delta_n) &&+tr_{n+1}^2\\
\gamma_{n+1} &= s(\tfrac{1}{4}\beta_n+\tfrac{1}{2}\gamma_n) &&+2tr_{n+1}p_{n+1}\\
\delta_{n+1} &= s(\tfrac{1}{4}\beta_n+\tfrac{1}{2}\delta_n) &&+2tq_{n+1}r_{n+1}
\end{aligned}$$

where

$$\begin{aligned}
p_{n+1} &= \lambda_n+\tfrac{1}{4}\beta_n+\tfrac{1}{2}\gamma_n\\
q_{n+1} &= \mu_n+\tfrac{1}{4}\beta_n+\tfrac{1}{2}\delta_n\\
r_{n+1} &= \alpha_n+\tfrac{1}{2}\beta_n+\tfrac{1}{2}\gamma_n+\tfrac{1}{2}\delta_n
\end{aligned}$$

and are the genotype frequencies in the $(n+1)$th generation.

From these equations it follows that

$$\begin{aligned}
4p_{n+2}-2sp_{n+1}-sp_n &= u(s+4tu)\\
4q_{n+2}-2sq_{n+1}-sq_n &= v(s+4tv) \qquad \text{(vii)}\\
4r_{n+2}-2sr_{n+1}-sr_n &= 8tuv
\end{aligned}$$

where u and v are the (constant) frequencies of the genes a and b (assuming no mutation). Solving these difference equations gives the distribution at any generation in terms of the initial distribution.

$$\begin{aligned}
p_n &= A_1\epsilon_1{}^n+B_1\epsilon_2{}^n+u(s+4tu)/(1+3t) &&\to u^2+uvs/(1+3t)\\
q_n &= A_2\epsilon_1{}^n+B_2\epsilon_2{}^n+v(s+4tu)/(1+3t) &&\to v^2+uvs/(1+3t)\\
r_n &= A_3\epsilon_1{}^n+B_3\epsilon_2{}^n+tuv/(1+3t) &&\to 2uv-2uvs/(1+3t).
\end{aligned}$$

The constants A_i, B_i are determined by the initial mating type frequencies. ϵ_1 and ϵ_2 are the roots of

$$4z^2-2sz-s = 0 \qquad \text{(viii)}$$

and hence $|\epsilon_i|<1$ for $|s|\leqslant 1$.

The form of the limiting or equilibrium frequencies indicates the departure from the random mating population (u^2, v^2, $2uv$). The equilibrium distribution is dependent only on the initial gene frequencies.

For a given value of s the equilibrium point, as represented in a trilinear diagram based on an equilateral reference triangle, lies on the parabola $\frac{1}{2}sr = t(4pq-r^2)$. Approach to this equilibrium parabola is along a straight line perpendicular to the base of the reference triangle from the initial point. The distance from the equilibrium decreases geometrically each generation.

This approach to equilibrium is very similar to that of mixed selfing and random mating with no selection (Hayman, 1953). We may also expect the approach under selection to be qualitatively similar for the genotypes in the two cases and so the general remarks by Hayman (1953, 4.2) should also apply here.

The mating types follow a much more complicated approach to equilibrium than the genotypes, being determined by a seventh order linear difference equation compared with the second order equation (vii) for the genotypes. Its characteristic equation contains equation (viii) as a factor. The equilibrium frequencies depend only on the gene frequencies u and v.

7. SUMMARY

The equilibrium is investigated of a large population under a system of mixed sib and random mating with selection against the homozygotes. Diagrams are produced giving the types of equilibrium and comparing this system with a system of mixed selfing and random mating.

Acknowledgment.—Thanks are due to Miss E. Stevenson of this Department for computing the graphs.

8. REFERENCES

HAYMAN, B. I. 1953. Mixed selfing and random mating when homozygotes are at a disadvantage. *Heredity*, 7, 185-192.

HAYMAN, B. I., AND MATHER, K. 1953. The progress of inbreeding when homozygotes are at a disadvantage. *Heredity*, 7, 165-183.

RECURRENT SELECTION

I. THE DETECTION OF OVERDOMINANCE

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1. INTRODUCTION

THE results of regression analyses have been widely used on many occasions to draw conclusions about the genetic basis of quantitative characters. In recent years much theoretical work has been published dealing with the application of this form of analysis to the determination of the average dominance of genes controlling such characters. Most of this theory refers to the analysis of diallel crosses (see Hayman, 1954) or to the relationship between inbred line performance and the performance of the crosses derived from such lines (Hull, 1952; Comstock and Robinson, 1952). Some attempt has been made to apply this technique to cases where both parents and progeny are heterozygous (Dickinson and Jinks, 1956).

A modified version of recurrent selection as suggested by Hull (1945) has been tried out experimentally on laboratory animals by Bell, Moore and Warren (1955), Rasmuson (1956) and Bowman (1958). This method of selection is designed primarily to exploit overdominance. At present there is no method of estimating overdominance in outbred populations. Consequently any analysis which can estimate the average dominance, or detect overdominance of genes controlling the character under selection would be valuable. An attempt has been made here by means of regression analyses to show one way in which overdominance can be detected in recurrent selection programmes as applied to animals. The analyses are essentially an extension to random bred individuals of the constant parent regression technique as applied to inbred lines by Hull (1952).

2. THEORETICAL ANALYSIS

The plan of recurrent selection as applied to animals has been outlined by Bell, Moore and Warren (1955), and the system of mating under consideration here is shown in fig. 1. In such a scheme two relationships are of particular interest. (i) The covariance between, and regression of, a sire's testcross progeny mean performance and his full sister mean performance. This is important in cases where the character under selection can only be measured in females, or where selection of heterogeneous strain progeny is carried out before their performance for the character can be measured. (ii) The covariance

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between, and regression of, a sire's testcross progeny mean performance and his pure strain progeny mean performance.

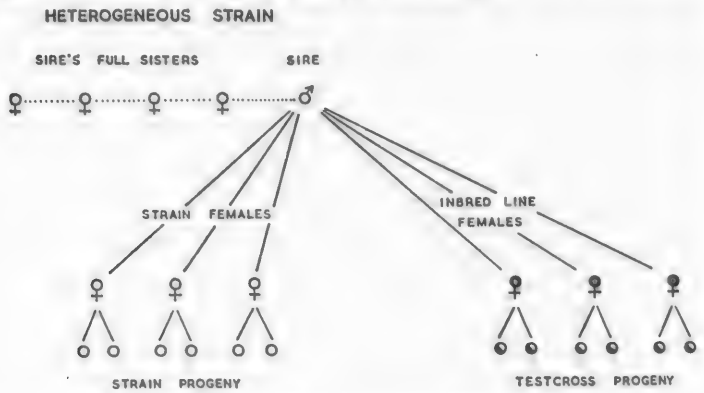

FIG. 1.—System of mating in a recurrent selection programme.

In the theory which follows a number of assumptions have been made. They are:

(*a*) Diploid segregation.
(*b*) No multiple allelism.
(*c*) Independent action of non-allelic genes.

Consider a single locus with two alleles, A_1 and A_2, such that the gene frequencies are p and q respectively, and such that $p+q = 1$.

TABLE 1

Genotypes and frequency of parents and progeny

Let $P = p^2$; $H = 2pq$; $Q = q^2$

Family type		Frequency of parent family	Genotype and frequency of progeny		
			A_1A_1	A_1A_2	A_2A_2
1.	$A_1A_1 \times A_1A_1$	P^2	P^2	...	...
2.	$A_1A_1 \times A_1A_2$	2PH	PH	PH	...
3.	$A_1A_1 \times A_2A_2$	2PQ	...	2PQ	...
4.	$A_1A_2 \times A_1A_2$	H^2	$\frac{1}{4}H^2$	$\frac{1}{2}H^2$	$\frac{1}{4}H^2$
5.	$A_1A_2 \times A_2A_2$	2HQ	...	HQ	HQ
6.	$A_2A_2 \times A_2A_2$	Q^2	...	...	Q^2
		Sums	$(P+\frac{1}{2}H)^2 = p^2$	$2(P+\frac{1}{2}H)(Q+\frac{1}{2}H) = 2pq$	$(Q+\frac{1}{2}H)^2 = q^2$

In a random mating population corresponding to the strain population in the scheme above there will be six family types. The frequencies of these types together with the genotype and frequency of their progeny are shown in table 1.

Using the notation of Mather (1949) let the phenotypic values of the three genotypes be:

A_1A_1	A_1A_2	A_2A_2
$+d$	h	$-d$

and then the mean value of the strain progeny

$$= d(p-q)+2pqh$$

and the variance

$$= 2pq[d-h(p-q)]^2+4h^2p^2q^2.$$

In a random mating population the mean and variance of a sire's full sisters will be the same as the mean and variance of his strain progeny. In a recurrent selection programme the genotype of the inbred tester will be either A_1A_1 or A_2A_2. Suppose the tester is A_1A_1. Then the mean values of the sire's sisters and the sire's testcross progeny are as shown in table 2 and table 3 respectively. The products of values of sisters × testcross progeny are shown in table 4.

TABLE 2

Mean value of a sire's sisters

Family type . .	1	2	3	4	5	6
Mean value of sire's sisters	d	$\frac{1}{2}d+\frac{1}{2}h$	h	$\frac{1}{2}h$	$-\frac{1}{2}d+\frac{1}{2}h$	$-d$

TABLE 3

Mean value of a sire's testcross progeny

	Sire genotype		
	A_1A_1	A_1A_2	A_2A_2
Mean value of testcross progeny			
Tester A_1A_1	d	$\frac{1}{2}d+\frac{1}{2}h$	h
Tester A_2A_2	h	$\frac{1}{2}h-\frac{1}{2}d$	$-d$

TABLE 4

Products of values of sisters × testcross progeny
Tester A_1A_1

Family type	Sire genotype		
	A_1A_1	A_1A_2	A_2A_2
1	d^2	...	...
2	$\frac{1}{2}d^2+\frac{1}{2}dh$	$\frac{1}{2}d^2+\frac{1}{2}dh+\frac{1}{2}h^2$	...
3	...	$\frac{1}{2}dh+\frac{1}{2}h^2$	...
4	$\frac{1}{2}dh$	$\frac{1}{4}dh+\frac{1}{4}h^2$	$\frac{1}{2}h^2$
5	...	$-\frac{1}{4}d^2+\frac{1}{4}h^2$	$-\frac{1}{2}dh+\frac{1}{2}h^2$
6	...	...	$-dh$

The uncorrected mean products = products × frequency

$$= (P^2+\tfrac{3}{4}PH-\tfrac{1}{4}HQ)d^2+(PH+\tfrac{1}{4}H^2+PQ-\tfrac{1}{2}HQ-Q^2)dh + (\tfrac{1}{4}PH+PQ+\tfrac{1}{4}H^2+\tfrac{3}{4}HQ)h^2.$$

The correction factor to obtain the covariance from the uncorrected mean products is the product of the mean value of sisters × mean value of testcross progeny. Converting p and q to P and Q, the mean value of sisters

$$= (p-q)d+2hpq.$$

Now $P-Q = p-q$.

Therefore mean value of sisters $= d(P-Q)+hH$.

The mean value of testcross progeny $= (p^2+pq)d+(pq+q^2)h$. Now $(P+\tfrac{1}{2}H) = p$.

Therefore mean value of testcross progeny

$$= dp+hq = d(P+\tfrac{1}{2}H)+h(Q+\tfrac{1}{2}H).$$

The product of the means

$$= (P^2+\tfrac{1}{2}PH-\tfrac{1}{2}HQ-PQ)d^2+(1\tfrac{1}{2}PH+\tfrac{1}{2}H^2-Q_2+PQ-\tfrac{1}{2}HQ)dh + (HQ+\tfrac{1}{2}H^2)h^2.$$

Then the subtraction of the correction factor from the sum of cross-products leaves the covariance, which can be shown to equal

$$d^2(\tfrac{1}{2}pq)+dh(-p^2q)+h^2(\tfrac{1}{2}pq[p-q]) = \tfrac{1}{2}pq[d^2-2pdh+(p-q)h^2]. \quad (1)$$

Hence the covariance of a sire's sister mean performance with his testcross progeny mean performance is:

when tester is $A_1A_1 = (1)$

when tester is $A_2A_2 = \tfrac{1}{2}pq[d^2+2qdh+(q-p)h^2]$. (2)

By a similar method of calculation it can be shown that the covariance of a sire's testcross progeny mean performance with his strain progeny mean performance is the same as (1) and (2) above when the tester is A_1A_1 and A_2A_2 respectively. The variance of a sire's strain progeny is the same as the variance of his sisters in the absence of selection. The conclusions about the covariance and regression which follow can therefore be applied to both relationships (i) and (ii).

The regression values for different values of p and q and for different values of h (various dominance levels) when $d = 1$ have been calculated and are given in tables 5 and 6. In cases where h has a value other than zero, dominance or overdominance is assumed to be in the direction of higher values, and p is the frequency of A_1 the allele that has the higher homozygote.

I am grateful to Dr Alan Robertson and Dr D. S. Falconer for showing me and allowing me to reproduce here an alternative method of deriving formulæ (1) and (2).

This method depends on knowing how breeding values and average effects of genes can be expressed in terms of the d and h notation of Mather (1949). The necessary interrelationships are given by Falconer (1959). Again let p and q be the gene frequencies of A_1 and A_2 in the

TABLE 5

Regression of a sire's testcross daughters on his sisters
Tester A_1A_1 ("high")

Values of p	$d = 1$ $h = 0$	$d = 1$ $h = 1$	$d = 1$ $h = 0{\cdot}5$	$d = 1$ $h = 2$	$d = 1$ $h = 1{\cdot}5$	$d = 1$ $h = 1{\cdot}25$	$d = 0$ $h = 1$
1·0	0	0	0	0	0	0	0
0·9	0·25	0	0·12	0·14	0·06	0	0·27
0·8	0·25	0	0·15	0·04	−0·02	−0·03	0·23
0·7	0·25	0	0·13	−0·03	−0·05	−0·04	0·16
0·6	0·25	0	0·12	−0·09	−0·06	−0·03	0·08
0·5	0·25	0	0·11	−0·08	−0·06	−0·03	0
0·4	0·25	0	0·10	−0·09	−0·06	−0·03	−0·08
0·3	0·25	0	0·10	−0·09	−0·06	−0·03	−0·16
0·2	0·25	0	0·09	−0·09	−0·05	−0·03	−0·23
0·1	0·25	0	0·10	−0·09	−0·05	−0·03	−0·27
0·0	0	0	0	0	0	0	0

p is frequency of A_1 in the strain. A_1 increases value.

strain from which the sires are taken; and let p' and q' be the corresponding gene frequencies in the tester strain. Let X be the average

TABLE 6

Regression of a sire's testcross daughters on his sisters
Tester A_2A_2 ("low")

Values of p	$d = 0$ $h = 0$	$d = 1$ $h = 1$	$d = 1$ $h = 0{\cdot}5$	$d = 1$ $h = 2$	$d = 1$ $h = 1{\cdot}5$	$d = 1$ $h = 1{\cdot}25$	$d = 0$ $h = 1$
1·0	0	0	0	0	0	0	0
0·9	0·25	0·45	0·35	−0·42	−0·29	0	−0·27
0·8	0·25	0·42	0·46	−0·11	0·09	0·25	−0·23
0·7	0·25	0·38	0·40	0·09	0·23	0·31	−0·16
0·6	0·25	0·36	0·36	0·20	0·28	0·32	−0·08
0·5	0·25	0·33	0·33	0·25	0·29	0·32	0
0·4	0·25	0·31	0·31	0·27	0·29	0·30	0·08
0·3	0·25	0·29	0·29	0·27	0·29	0·29	0·16
0·2	0·25	0·28	0·28	0·27	0·27	0·28	0·23
0·1	0·25	0·26	0·30	0·26	0·26	0·26	0·27
0·0	0	0	0	0	0	0	0

p is frequency of A_1 in the strain. A_1 increases value.

effect of the gene substitution in the pure strain, and X' the average effect of the gene substitution in the tester strain.

Now the breeding values of the three possible genotypes (a) when the sires are mated in their own strain, and (b) when they are mated in the tester strain, can be expressed as deviations from the population

means. These are shown in table 7. The expected values of daughters are half the appropriate breeding values given in columns (*a*) and (*b*). The covariance of sires' strain daughters with testcross daughters is therefore obtained by multiplying the frequency by $\frac{1}{2}$ the breeding value in column (*a*) by $\frac{1}{2}$ the breeding value in column (*b*) and summing. This gives

$$XX'[p^2qq'+\tfrac{1}{2}pq(q-p)(q'-p')+q^2pp']$$

which can be reduced to

$$\tfrac{1}{2}pqXX'$$

The average effect of a gene substitution expressed in the d and h notation is $X = d+h(q-p)$; so the covariance of the two sets of daughters becomes

$$\tfrac{1}{2}pq[d+h(q-p)][d+h(q'-p')] = \tfrac{1}{2}pq[d+h(q-p)][(d-h)]$$

if the tester is A_1A_1. This formula is equal to (1) above.

TABLE 7

Sires' breeding values in their own strain and in a tester strain

Genotype of sire	Frequency	Breeding values	
		(*a*) In own strain	(*b*) In tester strain
A_1A_1	p^2	$2qX$	$2q'X'$
A_1A_2	$2pq$	$(q-p)X$	$(q'-p')X'$
A_2A_2	q^2	$-2pX$	$-2p'X'$

Alternatively the covariance $= \tfrac{1}{2}pq[d+h(q-p)][(d+h)]$ if the tester is A_2A_2. This formula is equal to (2) above.

Now consider the sire's sisters. The expected value of any sister (or the mean of a number of sisters) is $\frac{1}{2}$ the breeding value $+\frac{1}{4}$ of the dominance deviation. The dominance deviation does not contribute to the covariance of sisters with daughters. So the covariance of sisters with testcross daughters is the same as that between strain and testcross daughters. This may be demonstrated as follows. The dominance of the sire's three genotypes are:

Sire genotype	Dominance deviation
A_1A_1	$-2q^2h$
A_1A_2	$2pqh$
A_2A_2	$-2p^2h$

These give the following additional terms to the covariance of strain sisters with testcross daughters (under (*b*) in table 7).

$$\tfrac{1}{4}hX'[-4p^2q^2q'+4p^2q^2(q'-p')+4p^2q^2p'] = hX'p^2q^2(-q'+q'-p'+p') = 0.$$

3. DISCUSSION AND PRACTICAL OBSERVATIONS

The exact relevance of applying conclusions based on calculations from single loci to characters of multigenic inheritance is unknown. For quantitative characters the overall regression observed is a summation of values for all loci controlling the character concerned. If the loci have unequal effects the observed regressions will be automatically weighted in favour of those with the largest effect. In practice it is likely that the inbred tester would be homozygous for the superior (or dominant to use the terminology of this paper) allele at the majority of loci. Superior here is defined as advantageous under conditions of natural selection. It has been suggested by Bowman (1958) that populations already at plateau level under selection for additive genetic variance would be suitable as the random mating populations in recurrent selection programmes. Plateau populations are theoretically expected to be homozygous for the superior allele at loci where gene action is additive. Therefore it seems reasonable to conclude that when plateau populations are used only loci where gene action is other than additive will contribute to the regressions being discussed. It must be stressed that no effort has been made to take account of epistasis and multiple allelism which could bias the value of the regressions.

With these considerations in mind it will be seen from examination of tables 5 and 6 that a negative regression will only be found in characters predominantly controlled by genes which are overdominant. This, however, does not imply the truth of the converse that if the regression is zero or positive, overdominance is absent. These conclusions regarding negative regressions are in agreement with those based on theoretical work on inbred lines by Hull (1952).

The value of the regression is dependent on and indicative of gene frequency. In practice, for characters which show overdominance, maximum performance would be achieved by obtaining 100 per cent. heterozygotes in the crossbred progeny. If the inbred tester is homozygous A_2A_2 then to obtain a negative regression the frequency of A_1 in the strain must be 0·7 or greater, except for very high degrees of overdominance. Improvement of hybrid mean performance will be obtained by changing the frequency of A_1 in the strain from $p = 0{\cdot}7$ to $p = 1{\cdot}0$. In that situation there will probably be less scope for improvement than if the tester is A_1A_1. A negative regression then means that the frequency of A_1 in the strain may be anywhere from $p = 0{\cdot}9$ to $p = 0{\cdot}1$. Maximum hybrid performance will be realised when $p = 0$ in the strain.

The performance of the strain itself will be affected by selection for hybrid performance. When the tester is homozygous recessive, strain performance will rise as hybrid performance increases, but when the tester is dominant the inverse relationship will hold. It may be that in the case where the tester is homozygous dominant natural selection would oppose any fall in strain performance caused by artificial selection for increased hybrid performance.

Two recurrent selection programmes have been carried out by Bowman (1958). In one case selection was for increased litter size in mice, and in the other for decreased number of abdominal chaetæ in Drosophila. Full details of these experiments will be described elsewhere. The mouse experiment had a design similar to that outlined in fig. 1, and was carried on for four cycles of selection. The regression of sires' crossbred progeny (X) litter size on their full sib (S) litter size was calculated for each cycle as well as a pooled estimate for the four cycles (see table 8). It can be seen that there is marked variation between cycles and from the pooled estimate in the value of the regressions. Since the mean litter size of S females varied

TABLE 8

Regression values of sires' crossbred progeny litter size on their full sisters' litter size, recurrent selection for litter size in mice

Cycle	Regression
1	$-0{\cdot}22 \pm 0{\cdot}11$
2	$0{\cdot}00 \pm 0{\cdot}17$
3	$-0{\cdot}22 \pm 0{\cdot}24$
4	$+0{\cdot}55 \pm 0{\cdot}15$
Pooled estimate cycles 1-4	$-0{\cdot}02 \pm 0{\cdot}08$

slightly between cycles it was decided to test the heterogeneity of the regressions with one another and at the same time adjust for the variation in mean. The method of Snedecor (1956) was used. There was no significant difference between adjusted means, but the separate cycle regressions were significantly heterogeneous.

The mean crossbred progeny litter size was increased by selection, and it seems probable that selection significantly altered the value of the regression at the same time. The fact that two of the regressions are negative, though the values are not significant, is slight evidence of overdominance. Further, if the regression has really changed from negative in the first three cycles to positive in the fourth, this indicates that dominant genes are probably at high frequency ($p = 0{\cdot}7$-$0{\cdot}9$) in the S strain. Such a situation offers ample scope for further improvement of hybrid performance.

The Drosophila experiment was carried on for fourteen cycles. Owing to the small numbers of individuals used, only three pooled estimates of the regression of sires' crossbred progeny bristle number on their pure strain progeny bristle number were calculated (see table 9). Though the regressions are not significant a marked decline in the observed value concomitant with a fall in the mean bristle number of both the crossbred and pure strain progeny was found. The inbred tester had previously been selected for low bristle number and was

consequently expected to be homozygous recessive at many loci. This considered in conjunction with the regression values suggests that overdominance for bristle number is negligible. Once again selection has probably altered the value of the regression, but in this case it is not so easy to make predictions about the changes in gene frequency in the pure strain.

Additive genetic variance can be more speedily exploited by mass or family selection methods within strains than by recurrent selection. It is therefore, useful to know from the first cycle of a recurrent selection programme whether there is any overdominance to exploit in the strain to be used for crossing to the inbred tester. It is seen that it is possible

TABLE 9

Regression values of sires' crossbred progeny bristle number on their pure strain progeny bristle number—recurrent selection for bristle number in Drosophila

Cycles	Regression
Pooled 1-5 . . .	$+0{\cdot}81 \pm 0{\cdot}60$
Pooled 6-10 . . .	$+0{\cdot}38 \pm 0{\cdot}89$
Pooled 11-14 . . .	$-0{\cdot}04 \pm 2{\cdot}03$

to detect overdominance, and that some predictions about expected response to recurrent selection can be made by the above approach.

4. SUMMARY

1. The values of two regressions—the relationship between the performance of a sire's testcross progeny and the performance of (i) his full sisters, or (ii) his pure strain progeny—are calculated by two methods in terms of gene frequencies and genotypic values for two alleles at a single locus.

2. It is pointed out that a negative regression can only be obtained when overdominance is present, though a positive or zero regression does not necessarily imply the absence of overdominance.

3. The possible use of these regressions in detecting overdominance and predicting progress in recurrent selection programmes is discussed.

4. Data on regressions calculated on two recurrent selection programmes, one for litter size in mice, and the other for bristle number in Drosophila are presented. With the theoretical findings in mind, a possible interpretation is placed on the regression values observed.

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5. REFERENCES

BELL, A. E., MOORE, C. H., AND WARREN, D. C. 1955. The evaluation of new methods for the improvement of quantitative characteristics. *Cold Spring Harbor Symp. Quant. Biol.*, *20*, 197-212.

BOWMAN, J. C. 1958. Recurrent selection : an experimental study with mice and Drosophila. Ph.D. Thesis, University of Edinburgh.

COMSTOCK, R. E., AND ROBINSON, H. F. 1952. Estimation of average dominance of genes. Chapter 30 of *Heterosis*, ed. by J. W. Gowen. Ames, Iowa State College Press. 552 pp.

DICKINSON, A. G., AND JINKS, J. L. 1956. A generalised analysis of diallel crosses. *Genetics*, *41*, 65-78.

FALCONER, D. S. 1959. *Introduction to Quantitative Genetics*. Oliver and Boyd, Edinburgh.

HAYMAN, B. I. 1954. The theory and analysis of diallel crosses. *Genetics*, *39*, 789-809.

HULL, F. M. 1945. Recurrent selection for specific combining ability in corn. *J. Amer. Soc. Agron.*, *37*, 134-145.

HULL, F. H. 1952. Recurrent selection and overdominance. Chapter 28 of *Heterosis*, ed. by J. W. Gowen. Ames, Iowa State College Press. 552 pp.

MATHER, K. 1949. *Biometrical Genetics*. Methuen and Co. Ltd., London.

RASMUSON, M. 1956. Recurrent reciprocal selection. Results of three model experiments on Drosophila for improvement of quantitative characters. *Hereditas* (Lund), *42*, 397-414.

SNEDECOR, G. W. 1956. *Statistical Methods*. Fifth Edition. Ames, Iowa State College Press. 534 pp.

NOTES AND COMMENTS

PHENOTYPIC LAG IN NEUROSPORA

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The study of heterocaryosis, where nuclei of more than one genotype share a common cytoplasm, has provided an insight into some of the mechanisms of nuclear and genic interaction (*e.g.* Beadle and Coonradt, 1944 ; Pontecorvo, 1946). A two component heterocaryon, represented as "*a*—*b*", *a* and *b* being the genotypes of the component nuclear types, will usually have a wild type phenotype. The large, vegetative spores (macroconidia) will be of three types ; namely, those having only the "a" type of nucleus, those having only the "b" type of nucleus, and those having both types of nucleus. These latter heterocaryotic conidia will tend to resemble the parent culture. The microconidia, being uninucleate, will be of only the first two types. They will, however, receive their cytoplasm from the parental (heterocaryotic) culture so initially the cytoplasm of such microconidia may differ from that carried by plants of similar genotype derived from a homocaryotic culture.

This paper reports the aberrant behaviour of uninucleate microconidia derived from a heterocaryon which, having the nuclei of one component, behaved like the other component.

1. MATERIALS

The two strains from which the heterocaryon was synthesised were a histidine, macroconidial, non-colonial, albino one (*h* ; *col-1*$^+$; *m*$^+$*su-m*$^+$; *al-2*) and a prototrophic, macroconidial, colonial, non-albino one (*h*$^+$; *col-1* ; *m su-m* ; *al-2*$^+$). *m* is peach microconidial (*pe*^{m}) (Barratt and Garnjobst, 1949) ; *su-m* is a modifier of *m* such that although *col-1* ; *m su-m*$^+$ is microconidial *col-1* ; *m su-m* is macroconidial (Grigg, 1958*b*) ; *h* is the histidine gene *K26his-3* (Mathieson and Catcheside, 1955) ; *al-2* is an albino gene (Beadle and Tatum, 1945) ; and *col-1* is the Y8743 *colonial-1* gene of Barratt and Garnjobst (1949).

Despite the macroconidial nature of the two parental homocaryons the heterocaryon was microconidial and made a spreading growth on minimal medium. At the particular nuclear ratio obtaining, namely, 1 *h* to 15-30 *h*$^+$ nuclei, *h*$^+$ was "dominant" to *h*, *su-m*$^+$ was "dominant" to *su-m*, *al-2*$^+$ was "dominant" to *al-2*, but the mutant gene *m* was "dominant" to its wild type allele (*m*$^+$). Two phenotypic effects of *col-1*, namely, inhibition of macroconidia formation and restriction of growth rate, showed different dominance relations, the first being "dominant" to the wild type and the second being "recessive" (Grigg, 1958*b*).

The histidine strain will not grow on "complete" media presumably because histidine uptake is inhibited by other amino acids present (Mathieson and Catcheside, 1955). Heterocaryons grew well on rich conidiating complete medium (Horowitz, 1947), and produced approximately 10^8 microconidia per slope.

2. RESULTS

In the course of an attempt to measure the nuclear ratio of the heterocaryon equal numbers of microconidia were plated (Grigg, 1958*a*, *b*) into a minimal medium and a similar medium supplemented with histidine (30γ/ml.). The method proved useless and gave an erroneous estimate of the nuclear ratio (Grigg, 1958*a*). Only one histidine colony was observed among over 6500 colonies which appeared on the supplemented medium. The others had the phenotype of the h^+ ; *col-1* ; *m su-m* ; *al-2*$^+$ strain. However, the numbers of these colonies which appeared on the supplemented medium was very different from those which appeared on the minimal medium. There was a considerable deficit on the minimal plates. Following this observation the plating experiment was repeated twelve times using fresh media each time. Similar results were obtained on each occasion. The data of a typical experiment are shown in table 1. Microconidia from a homocaryotic h^+ strain germinated equally well on the

TABLE 1

Mean number of colonies per Petri plate of medium following the plating of equal numbers of microconidia on minimal and on histidine supplemented medium. The values are the means of four replicates

Source of microconidia	Media	
	Minimal	Histidine
h^+—*h* heterocaryon .	6·5±2·3	46·5±3·0
h^+ homocaryon . . .	100·0±7·0	104·3±2·6

minimal and supplemented media, but microconidia from the heterocaryon germinated much better on histidine medium. In the thirteen plating experiments the number of + ; *col-1* ; *m su-m* ; + colonies which appeared on minimal medium ranged from one half to one twenty-fifth of the number observed on the supplemented medium. In some experiments a layer of histidine medium was poured over the minimal medium in which conidia had been plated four days previously. Additional colonies appeared on these double layer plates so that the final numbers equalled those on the supplemented plates. Those colonies which were visible at the time when the second layer of medium was poured over them had not commenced to conidiate so the additional colonies cannot be ascribed to the presence of pre-existing colonies. We infer that they arose from previously ungerminated spores.

Germination of the microconidia from the heterocaryon was as good on complete (Horowitz, 1947, conidiating complete) as on histidine medium (table 2). The equivalence of conidiating complete to histidine medium in aiding germination of these conidia may be contrasted with its failure, although it contains histidine, to support growth of the K26 strain.

Addition of growth factors other than histidine did not increase the number of colonies which appeared on minimal medium. It is concluded that the effect is specific to histidine.

The colony counts on minimal medium have a higher coefficient of variation than those of the " histidine " and " complete " groups. On the other hand the colony counts on the minimal plates containing microconidia from an h^+ had a coefficient of variation comparable to those of the supplemented groups. The variance tended to be high in all cases where there was a deficiency of colonies.

The requirements for histidine which a proportion of the conidia exhibited was a transient phenomenon and was limited to their initial germination. Once germinated the conidia grew at the h^+ rate. The colonies grown from these microconidia produced normal h^+ conidia as judged by the way in which samples from 20 colonies germinated equally well on minimal and histidine supplemented medium.

TABLE 2

Number of colonies per plate of medium following the addition of equal numbers of microconidia to minimal, histidine supplemented and complete media

Medium		
Minimal	Histidine supplemented	Complete
69	166	140
62	143	156
14	139	142
73	157	164
$\bar{x}$ 54·5±13·7	151·25±6·3	150·5±5·7
v 562·25	117·5	99·0

The uninucleate nature of microconidia reported by Barratt and Garnjobst (1949) was confirmed by examination of microconidia stained according to Huebschman (1952).

3. DISCUSSION

The observation that germination of h^+ microconidia from h^+—h heterocaryons is lower on minimal than on supplemented medium indicates that some factors are in low concentration or are entirely lacking in the cytoplasm of the freshly formed microconidium. To initiate synthesis of these factors by the h^+ nuclei, histidine must be supplied. We may conclude from this that somewhere along the synthetic pathway of the histidine factors there occurs an unbalanced or an irreversible reaction. If all the reactions along this pathway were completely reversible the depletion or absence of the histidine factors in the cytoplasm would change the relative reaction rates of reactants and products to result in increased histidine synthesis.

An alternative explanation is that some cytoplasmic factors, which require the presence of the h nuclei for their continued production, are carried over into the freshly formed conidia. These factors might reduce the ability of the conidia to germinate on the minimal medium. This seems less likely than the first alternative.

A phenomenon similar to this one in some respects involved the germination of macroconidia from some macroconidial heterocaryons (Grigg, 1958*b*). The histidine component in each of the macroconidial heterocaryons was the same (K26) as that in the microconidial heterocaryons. In a macroconidial heterocaryon in which the nuclear ratio in the culture of $h : +$ was 2 : 1 the heterocaryotic conidia germinated more slowly on minimal than on histidine medium. On the other hand when the nuclear ratio was 1 : 2 the macroconidia germinated equally well on both.

Both the microconidial and the macroconidial heterocaryons grew well on minimal or on complete medium. For reasons previously mentioned the histidine component of the heterocaryons does not grow on complete media despite the latter's content of histidine. It is perhaps puzzling then that complete medium aided the germination of the microconidia from the heterocaryon just as well as did histidine. Recently some partial back-mutants at the same histidineless locus have been found which show a similar pattern of growth requirements in that the growth rates are increased if either histidine or complete medium is added.

The observation that the percentage germination of partial revertants can be lower on minimal medium than on medium containing appropriate growth substances has importance in the planning and interpretation of mutation experiments. Any growth promoting substances present are released if conidia are killed (Grigg, 1958*a*). Mutagens may be often highly toxic and so the chance of pre-existing back-mutant nuclei germinating and being detected as colonies would be higher in the mutagen treated plates than in the control ones. As a result it might be difficult to decide whether the wild type colonies which arose following treatment of the conidia reflected the mutagenicity of the treatment or merely its toxicity.

4. REFERENCES

BARRATT, R. W., AND GARNJOBST, L. 1949. Genetics of a colonial microconidiating mutant strain of *Neurospora crassa*. *Genetics*, *34*, 351-369.

BEADIE, G. W., AND COONRADT, V. L. 1944. Heterocaryosis in *Neurospora crassa*. *Genetics*, *29*, 291-308.

BEADLE, G. W., AND TATUM, E. L. 1945. *Neurospora*. II. Methods of producing and detecting mutations concerned with nutritional requirements. *Am. J. Bot.*, *32*, 678-686.

GRIGG, G. W. 1958*a*. Competitive suppression and the detection of mutations in microbial populations. *Aust. J. Biol. Sc.*, *11*, 69-84.

GRIGG, G. W. 1958*b*. The genetic control of conidiation in a heterokaryon of *Neurospora crassa*. *J. Gen. Microbiol.*, *19*, 15-22.

HUEBSCHMAN, C. 1952. A method for varying the average member of nuclei in the conidia of *Neurospora crassa*. *Mycologia*, *44*, 599-604.

HOROWITZ, N. H. 1947. Methionine synthesis in *Neurospora*. The isolation of cystathionine. *J. Biol. Chem.*, *171*, 255-264.

MATHIESON, M. J., AND CATCHESIDE, D. G. 1955. Inhibition of histidine uptake in *Neurospora crassa*. *J. Gen. Microbiol.*, *13*, 72-83.

PONTECORVO, G. 1946. Genetic system based on heterocaryosis. *Cold Spring Harbor Symp. Quant. Biol.*, *11*, 193-201.

VARIATION OF CHIASMA FREQUENCY IN AND BETWEEN NATURAL POPULATIONS OF ACRIDIDAE

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1. INTRODUCTION

Given any significant heterozygosity the genetic stability and variability of populations must be affected by any variation of chiasma frequency that may occur (Darlington, 1939; Mather, 1943; Thoday, 1953). Natural populations of grasshoppers (*Myrmeleotettix maculatus* (Th.) and *Chorthippus parallelus* (Zett.)) have therefore been examined by analysis of metaphase chiasma frequencies in males to see whether such variation is to be found.

2. MATERIAL

The populations sampled were in the north and south of England (the Sheffield region, and the Ascot and New Forest regions respectively). The grasshoppers were collected in the early evening and their testes fixed within two hours of collection in Strong Flemming's solution. The sections were stained with crystal violet. Chiasmata were counted in five cells per individual and usually in eight individuals per sample.

3. RESULTS

(i) *Chiasma frequencies*

Though the experimental results are limited they seem to demonstrate that variation of chiasma frequency does occur in and between these natural populations. In both *Myrmeleotettix* and *Chorthippus* it was found (i) that there were significant differences between individuals within populations (compare Elliott, 1954), and (ii) samples from different areas had significantly different chiasma frequencies.

In *Myrmeleotettix* the two high altitude (over 1,000 feet) northern populations had lower chiasma frequencies (mean = 14·36) than the two southern populations which were at low altitudes (mean = 15·18). An analysis of the relevant data is given in table 1. These differences in chiasma frequency between areas might be related to climate: the high altitude northern populations were found in cooler, wetter areas than the southern populations.

Samples of *Myrmeleotettix* were also obtained from three medium and low altitude northern populations and their chiasma frequencies varied between those characteristic of the high altitude northern and those characteristic of the southern populations.

All the populations of *Chorthippus* were at low altitudes. Data were obtained from one northern population (two samples) and two southern populations (Ascot and New Forest). The samples from the north (mean = 14·54) had a significantly lower chiasma frequency ($P < 0{\cdot}01$) than those from the south (mean = 15·51).

* Formerly: The Zoology Department, Sheffield University.

It seems unlikely that the differences of chiasma frequency are directly caused by summer environmental differences for the following reasons :— (*a*) One population of *Chorthippus* was sampled both at mid-day and in the early evening. The two samples had similar chiasma frequencies. (*b*) Five populations (four *Myrmeleotettix* and one *Chorthippus*) were each sampled in

TABLE 1

Analysis of variance of number of chiasmata in the Myrmeleotettix *field samples from the high altitude northern and low altitude southern populations (means listed in table 2)*

Source	*n*	Mean square	P
Areas	1	52·8125	<0·01 †
Years	1	4·05	...
Years × areas	1	2·45	...
Between populations within areas	2	1·325	...
Residual interaction	1	3·725	...
Total between samples	6	10·9479	about 0·05 †
Between individuals within samples (error 2)	48	4·6880	<0·001 *
Total between individuals	54	5·3836	<0·001 *
Within individuals (error 1)	224	1·1268	...

* Compared with error 1. † Compared with error 2.

TABLE 2

Chiasma frequencies in some of the Myrmeleotettix *samples*

	Field samples			
	New Forest		High altitude North	
	1955	1956	1955	1956
Population 1	15·58	15·00	14·58	14·28
Population 2	...	14·95	14·20	14·40

	Greenhouse experiments (1956)					
	New Forest			High altitude North		
	Experiments			Experiments		
	1	2	3	1	2	3
Population 1	...	15·38	15·13	...	...	...
Population 2	...	...	...	14·50	14·43	14·13
Population 3	15·38	...	...	...	...	...

different months of the same summer. There was no significant difference between the samples collected earlier and those collected later in the summer. (*c*) Four populations were sampled in both 1955 and 1956 and there was no significant difference between years, though the summer of 1955 was hot and dry and that of 1956 very cold and wet. (*d*) Three experiments were carried out, two starting with adults and one with fourth instars, to test whether the differences of chiasma frequency between populations of *Myrmeleotettix* would still be evident if individuals from different populations were kept together in the same environment (a greenhouse in Sheffield) for a few days prior to the time when the chiasmata were actually formed. Analysis of variance showed that the difference between the high altitude and the southern populations was not affected and there was no significant difference between field and greenhouse samples. Table 2 summarises the data.

All these five tests suggest that the differences in chiasma frequency between areas are fairly stable. It seems reasonable to conclude that the southern populations of *Myrmeleotettix* and *Chorthippus* have a consistently higher chiasma frequency (and hence more genetic recombination) than certain of the northern populations.

(ii) *Supernumeraries*

Supernumerary chromosomes were found in some individuals in five out of the eight populations of *Myrmeleotettix* studied. Individuals with supernumeraries usually had one or two per cell and these chromosomes were never found paired with members of the " normal " complement at first meiotic metaphase (fig. 1).

Fig. 1.—1st meiotic metaphase. *Myrmeleotettix maculatus*. Magnification 1200. The rod-shaped sex chromosome and the supernumerary chromosome are seen lying off the metaphase plate.

Supernumerary chromosomes were present in all the southern samples of *Myrmeleotettix* but not in any of the samples from high altitude northern populations. These chromosomes were also found in a few individuals in two low altitude northern areas. Analyses showed that, within populations, those individuals with supernumeraries had significantly higher chiasma frequencies in the " normal " members of the chromosome complement than did those lacking supernumeraries. Further, no significant difference in chiasma frequency between the high altitude northern areas and the

southern areas was demonstrable in *Myrmeleotettix* when individuals with supernumeraries were left out of the analysis. It seems most likely that the supernumerary chromosomes have the effect of raising chiasma frequency in the other chromosomes (compare Mather, 1939) and that their presence is at least partly responsible for the higher chiasma frequencies of the southern populations of *Myrmeleotettix*. (It must be noted that any chiasmata between or within supernumeraries were ignored throughout the analyses.)

Supernumeraries were not found in *Chorthippus*, yet there were differences in chiasma frequency between the samples from the north and those from the south in this species, that were similar in magnitude to those found in *Myrmeleotettix*. It would seem that both species may have developed higher chiasma frequencies in the south and lower in parts of the north but that the regional differences have been produced by different mechanisms. That both species showed similar differences suggests that higher chiasma frequencies may be advantageous in the south (or lower frequencies advantageous in the north). It is therefore possible that supernumeraries were selected in the southern populations of *Myrmeleotettix* primarily because they affect chiasma frequency, or eliminated from the northern high altitude populations because they raise chiasma frequency.

4. CONCLUSION

The results reported are not extensive, but they do suggest that further study of chiasma frequency and supernumerary frequency in natural populations of these species would be well worth while. If such a study could be combined with ecological study to determine for example whether chiasma frequency in marginal populations (such as the high altitude populations of *Myrmeleotettix* may be) differs consistently from that in the more central populations (compare Carson, 1955) new understanding of natural populations might result.

Acknowledgments.—I would like to thank Dr J. M. Thoday for suggesting this study, Professor L. E. S. Eastham, and all others who helped. I also acknowledge the receipt of Research Fund Grant number 206 from Sheffield University.

5. REFERENCES

CARSON, H. L. 1955. Variation in genetic recombination in natural populations. *J. cell. comp. Physiol.*, *45*, Suppl. 2, 221-236.

DARLINGTON, C. D. 1939. *The Evolution of Genetic Systems*. Cambridge University Press.

ELLIOTT, C. G. 1954. Variation in chiasma frequency in natural populations. *Proc. 9th International Congress of Genetics*, part II, 689-690.

MATHER, K. 1939. Competition for chiasmata in diploid and trisomic maize. *Chromosoma*, *1*, 119-129.

MATHER, K. 1943. Polygenic inheritance and natural selection. *Biol. Rev.*, *18*, 32-64.

THODAY, J. M. 1953. Components of fitness. *Symp. Soc. Exp. Biol.*, 7, 96-113.

REVIEWS

OUTLINE OF HUMAN GENETICS. By L. S. Penrose. London: Heinemann. 1959. Pp. 146+xii. 12s. 6d.

As Professor Penrose says in opening his introduction " The study of human genetics is of interest to everyone "—and not only from the rather general point of view of trying to understand ourselves and how we came to be what we are, but also nowadays as an essential to the adequate appraisal of, to take but one example, the consequences for mankind of the steadily growing amount of ionising radiations to which we are exposed by developments in industrial, medical and military practice. *Outline of Human Genetics* sets out to introduce the facts of human genetics in such a way as to " be understandable by the majority of readers, whether or not they have previous knowledge of the subject ". All will laud this aim, and I believe that Professor Penrose has provided an account which the public can read with interest, enjoyment and profit. It is, of course, difficult for a professional geneticist to assess the appeal which such a book will make to the general reader, but the dust cover conveys Dr Bronowski's assurances on this point. I can only add the hope that it will be widely read : the general public has a great deal to gain from it—and so have geneticists from the wider understanding of their science that should then follow. This having been said, Professor Penrose will perhaps forgive me if here and there I am critical of the balance he achieves and the picture he paints.

The treatment is unusual. There is no attempt to lay down a formal framework and then build the appreciation of human genetics round it. Rather the theory is developed, somewhat piecemeal, out of miscellaneous observations. " Genes and Populations " comes before " Association and Linkage ", and both before " The Interplay of Heredity and Environment ". And there is no single comprehensive account of chromosome behaviour and its consequences. This would hardly do for instructing a class of students but the result is very readable and may well be just the kind of treatment to appeal to the general reader. The illustrations are good, even if perhaps a little sparse, and some of the pedigrees are noteworthy in their information and interest. Surely, however, a diagram or two of chromosome behaviour would have helped : the table which sets out to illustrate the effects of crossing-over will puzzle many readers, the more so as it is divorced from any explanatory text. Diagrammatic treatment could have been more informative, and much more nearly self-explanatory.

Characters showing continuous variation are mentioned in several places, but they nowhere achieve a prominence commensurate with their importance in human populations. Professor Penrose gives his reasons for this early in the book where (on page 7) he says in effect that as they do not " correspond to genetical units " definite " answers to the urgent questions about the inheritance of such characteristics as health and intelligence " can be much less readily given. All the same, our knowledge of these characteristics is not negligible, and if the answers are more involved they are not any the less important. Furthermore brevity of mention can easily become confused with insignificance, especially in the lay mind.

Urgent questions must be explored even if their complexity makes answers less confident and less precise. Fair treatment is not necessarily final treatment, and the reader can be given the means of making up his own mind as to how far he is prepared to pursue an argument or accept an opinion.

This is not to say that Professor Penrose avoids all controversial matters or confines himself to stating generally accepted views. Indeed he warns his readers that in the last chapter, on " Eugenics and Dysgenics " his own opinions predominate. Much in this chapter will be accepted as sound and salutary, but much strikes at least one reader as open to very sharp challenge. For example, we read on page 119 that " variability in a population is biologically desirable because it provides the necessary energy for adaptation and evolution ", the variability referred to being by implication not merely genetic but also much of it of the pathological or near pathological kind to which a major part of the book has been devoted. Some kinds of genetic variability are unquestionably the basis of adaptation in plant and animal species. But are not other kinds—the lethals and the crippling mutations—merely the outcome of unavoidable breakdown in the genetic materials and genetic system ? And does any kind of genetic variability have the same importance for progressive adaptation in man as in other species ? This is surely a matter for discussion rather than assertion : here above all places definite and final answers are hardly possible at present.

Then again, the discussion of intelligence and fertility is hinged on a single gene model which results in the conclusion that " the genetic backbone of the population is, in fact, the despised mentally inferior tenth ". The conclusion is of course implicit in the assumption and the model does serve to show how complex and surprising in their consequences genetical situations can be. But when it leads to such a conclusion can anyone take the model seriously ? Does Professor Penrose really believe that it fairly, or even usefully, represents, albeit at a simplified level, the situation with respect to intelligence and fertility ?

The significance of variability in man and the intricacies of his population genetics have provided many arguments and they will provide many more. They are indeed matters of significance not merely to geneticists but to us all, and all should be aware of the questions at issue, the consequences of error and drift, and the pressing need for more research and more knowledge. Professor Penrose is doing a valuable service in presenting human genetics to the general reader.

KENNETH MATHER.

THE MECHANISM OF EVOLUTION. 2nd edition. By W. H. Dowdeswell. London: Heinemann. 1958. Pp. 115. 7s. 6d.

NATURAL SELECTION AND HEREDITY. Revised 3rd impression. By P. M. Sheppard. London: Hutchinson. 1959. Pp. 212. 10s. 6d.

Dowdeswell gives us an account of what we may call phenotypic evolution. The latest evidence of variation and selection of phenotypes in nature, and also the study of the fossil record, are considered in relation to what Darwin and others have said about them. Within these limits the account is admirable. But is it right to set such limits ? Since the word " mechanism " is used, it is strange that the mechanism of heredity and its variation is not roundly presented. Is that not a part of the mechanism of

evolution? Darwin thought so and would have said something about it if he could have done so.

By implication, of course, something is said but it is not too reassuring. What are these "genes" that suddenly crop up? (p. 14). Perhaps Mendel invented them. Ought we to talk about the "fact" of dominance (p. 15)? Can polyploids be understood without any explanation of diploids? (p. 21). Can autogamy in *Paramecium* (p. 43) be understood without a discussion of meiosis? This book is intended for schools. But if beginners are shown their subject dismembered, and with its parts in the wrong order, will they ever unlearn their mistake?

The same criticisms apply in part to the new impression of Dr Sheppard's book. Great advances will no doubt continue to be made from studying the working of natural selection as naturalists have studied it in the past. But what about the great questions of reproduction, hybridity, fertility and sterility which concerned Darwin so much, questions to which we have now found answers? More and more advances will fail to be made if we pretend that naturalists need not worry about the answers to such questions. Or if we imagine that natural selection does not affect them.

C. D. DARLINGTON.

THE CHEMICAL BASIS OF DEVELOPMENT. Ed. by William D. McElroy and Bentley Glass. Baltimore: John Hopkins Press; London: Oxford University Press. 1959. Pp. 934. £6.

CELL, ORGANISM AND MILIEU. Ed. by Dorothea Rudwick. New York: Ronald Press. 1959. Pp. 326. $8.

DEVELOPMENTAL CYTOLOGY. Ed. by Dorothea Rudwick. New York: Ronald Press. 1959. Pp. 215. $7.

These three symposia overlap in interest and in all of them some papers are of concern for genetics.

The Chemical Basis of Development deals chiefly with animal biochemistry but there is a discussion of enzyme induction in *Escherichia* and two papers on slime moulds. Gall gives a valuable account of polytene and lampbrush chromosomes and Swift analyses the relations of RNA with the nucleolus in amphibia. The most important contribution is Hadorn's account of the tissue and phase specificity of gene action, especially in regard to the rosy gene in *Drosophila*. This is surely to be related to the observations of chromomere variation in polytene chromosomes. Both types of observation indicate that one gene may be having effects which vary in different cells at the same time, and at different times in the same cell.

There is no general or biological discussion of the chemical basis development but there is a summation of 12 pages by Paul Weiss and a summary of 68 pages by Bentley Glass and there are copious discussions of separate papers.

Cell, Organism and Milieu ranges from the effects of the RNA of live microsomes in changing fibroblasts into neuron-like cells in tissue culture, on the one hand, to the action of hormones of the auxin complex and their substitutes in stimulating fruit development at various stages, on the other hand. Both these types of investigation have wide biological implications.

Developmental Cytology, like the other symposia, deals mainly with peripheral problems which do not do justice to the title. Beermann's discussion of polytene structure and activity is, however, most appropriate. Lehninger's views on reactions within and between mitochondria and other particles

break new gound. So also does Wettstein's distinction between the effect of mutations within plant plastids in upsetting the mature plastid while nuclear gene mutations upset their earlier development. Numerous electron microscope photographs and excellent diagrams add to the value of this study. C. D. D.

BRITISH MEDICAL BULLETIN 15 (2) BLOOD GROUPS. British Council: Med. Dept. 1959. Pp. 89-174. 20s.

BRITISH MEDICAL BULLETIN 15 (3) CURRENT VIRUS RESEARCH. British Council: Med. Dept. 1959. Pp. 175-250. 20s.

The symposium on blood groups covers the whole of the known—and still actively ramifying—structure of blood group research. From the central enquiry into pedigrees discussed by Race, the clinical, immunological, biochemical and evolutionary themes radiate. The new problems of selection and the stability of polymorphism are dealt with by Fraser, Roberts and Sheppard. Geneticists are likely to be first impressed by the growing importance of these genetical treatments for medicine. But it is no less clear that the techniques of human genetics are here beginning to compete favourably with the experimental techniques on which fundamental advances in genetics have hitherto depended.

In the bulletin on virus research two articles are particularly important for genetics. Fenner uses the model of myxomatosis to demonstrate the interaction of selection on host and virus in the history of this disease. Burnet interprets the evolution of virulence and attenuation (especially in influenza) in terms of recombination as well as of mutation and selection. C. D. D.

HISTORY OF BIOLOGY. By F. S. Bodenheimer. London: Dawson. 1958. Pp. 465. 42s.

Biology is now in the process of acquiring the unity which its name has foretold since 1800. It is natural therefore to think that one man may attempt to write a single history of the subject. But the subject, we find, is growing together from many kinds of study which have not yet converged. It is still tied up with such a wide range of ideas and conflicts of ideas that its history has little unity. And past attempts show only too clearly that the writing of its history demands the meeting of several minds.

Allowing for this difficulty it is hard to see the shape or purpose in Dr Bodenheimer's book.

Its three parts—introduction, narrative and collection of original quotations—are of unequal merit. The introduction, described as " pererga and paralipomena " in other words, frills (79 pages), contains personal opinions and ideas, some of them of interest. The narrative, described as " a short factual history " (65 pages) is indeed a short conventional history. It seems to be a collection of notes made over a period of thirty years and pieced together with little connection. It is trite and tedious. The original quotations (309 pages) are again some of them of interest. These, like the two short lists of references to works by historians, are in no order, chronological or alphabetical: again badly pieced together.

The publisher does not seem to have helped or guided the author where he might have done. There is no list of plates, no subject index, and no page references to the three lists of contents. These would have been of more use than the frills. C. D. D.

BOOKS RECEIVED

THE PHYSICAL FOUNDATION OF BIOLOGY. By Walter M. Elsasser. London, New York, Paris, Los Angeles: Pergamon Press. 1958. Pp. 219. 30s.

MITOGENESIS. Report: Developmental Biology Conference Series 1956., Ed. by H. S. Ducoff and C. F. Ehret. University of Chicago Press. 1959. Pp. 114. 24s.

ENVIRONMENTAL INFLUENCES ON PRENATAL DEVELOPMENT. Report: Developmental Biology Conference Series, 1956. Ed. by Beatrice Mintz. University of Chicago Press. 1959. Pp. 87. 22s. 6d.

THE PRINCIPLES OF HUMAN EXPERIMENTAL TECHNIQUE. By W. M. S. Russell and R. L. Burch. London: Methuen. 1959. Pp. 238. 30s.

THE HEALING VOICE. By Dr A. Philip Magonet. London: Heinemann. 1959. Pp. 205. 18s.

ABSTRACT BIBLIOGRAPHY OF FRUIT BREEDING AND GENETICS TO 1955. RUBUS AND RIBES—A SURVEY. By R. L. Knight and Elizabeth Keep. Commonwealth Agricultural Bureaux, Farnham Royal. Pp. 254. 45s.

PRACTICAL BIOLOGY FOR ADVANCED LEVEL, MEDICAL AND INTERMEDIATE STUDENTS. Vol. II. PRACTICAL BOTANY. By C. J. Wallis. London: Heinemann. 1958. Pp. 173. 17s. 6d.

BIOLOGICAL LABORATORY DATA. By L. J. Hale. Methuen, London. 1958. Pp. 132. 15s.

GENETICAL SOCIETY OF GREAT BRITAIN

ABSTRACTS of Papers read at the HUNDRED AND THIRTIETH MEETING of the Society held on 16th and 17th JULY 1959, in DUNDEE under the auspices of the Scottish Horticultural Research Institute

PHENOPLOIDY AND THE DISTRIBUTION OF EUROPEAN AND BRITISH BLACKBERRIES (*RUBUS FRUTICOSUS AGG.*)

G. HASKELL

Scottish Horticultural Research Institute, Invergowrie, by Dundee

The changes in proportion of the various sub-sections of apomictic polyploid *Rubus* across Europe are demonstrated : the north-western countries have a higher frequency of simple forms of blackberries, whereas south-eastern countries, such as the Caucasus and Hungary, have a higher proportion of complex types.

The distribution of the 344 "species" of blackberries in Britain is analysed for the five sub-sections, being based upon Watson's posthumously published data. The centre of diversity in south-east England is demonstrated ; this may be related to the proximity with the Continent, or to greater numbers of botanists in this area. The general pattern of distribution appears to reflect the limits of glaciation, with the diversity of species north of the original limits of glaciation falling off very rapidly. The relation between taxonomy and scarcity indicates that the sub-sections with the simpler types are less restricted in their distribution than the sub-sections of complex forms.

The relative abundance of *Rubus* species in Great Britain will be demonstrated. The number of species plotted against the vice-comital frequency shows a log series. The number of species per vice-county grouped in geometric X_3 classes shows a close fit with the theoretical Poisson distribution.

The basis for the variation in these apomictic polyploid *Rubi* will be considered in relation to the cytological origin of the apomictic mechanism.

PROBLEMS IN BREEDING BRUSSELS SPROUTS (*BRASSICA OLERACEA GEMMIFERA*) AT MYLNEFIELD

C. NORTH and W. G. PRIESTLEY

Scottish Horticultural Research Institute, Invergowrie, by Dundee

The aim of the Brussels sprout breeding work is to produce uniform strains adapted to the Scottish climate. This is being attempted by selection and controlled pollination to combine the desirable characters of several strains, *e.g.* large, firm and dark-coloured sprouts and resistance to lodging.

Some of the practical difficulties of this work will be discussed and the following special techniques used at Mylnefield will be described : (1) the routine technique for hand pollination, (2) the use of small honey bee colonies in artificially heated hives, (3) the use of ramets of selected clones, (4) the objective measurement of "sprout" firmness mechanically.

The possible use of male-sterile plants to produce F_1 hybrid seed of Brussels sprouts on a commercial scale will be discussed. Male sterile plants have been found, and the inheritance of this character is being studied. Preliminary experiments on the artificial induction of male sterility by chemical sprays have given promising results.

GENETICS AS AN AID TO THE CONTROL OF PLANT VIRUS INFECTION

C. H. CADMAN
Scottish Horticultural Research Institute, Invergowrie, by Dundee

The production of varieties of plants which resist, or are immune from, infection by viruses, or which escape infection because unpalatable to virus vectors, seems to offer the most lasting solution to the problem of controlling virus spread in the field. These possibilities seem not to have been fully explored by geneticists, particularly in the raspberry, where genes controlling immunity or resistance to aphid- and soil-borne viruses exist among commercial varieties.

SOME ASPECTS OF PLANT DISTRIBUTION IN BRITAIN

J. R. MATTHEWS
Department of Botany, Aberdeen University

Since Britain is a continental island of relatively recent origin, it is not surprising that its flora is closely allied to the flora of the European mainland, even although it is no more than an impoverished portion of that flora. Nevertheless, the time factor would appear to have been adequate to allow of the production of a number of endemic species such as *Primula scotica*, *Cochlearia micacea* and others. Apart from endemics, the bulk of our native flora may be resolved geographically into three major components, each of which, however, is capable of sub-division. These several elements will be briefly described and it will be shown that what is applicable to the flora of Britain as a whole may apply also to a more limited area such as Perthshire.

SOME PROBLEMS IN BREEDING STRAWBERRIES RESISTANT TO RED CORE DISEASE (*PHYTOPHTHORA FRAGARIAE* HICKMAN)

R. D. REID and I. G. MONTGOMERIE
Scottish Horticultural Research Institute, Auchincruive, Ayr

Breeding for control of this disease has been proceeding for over 25 years. In applied work, testing is by field and bench methods. Susceptibles, when selfed, never produce resistants. Proportion of seedlings resistant in first test usually shows progressive increase of susceptibility in subsequent years. Selections resistant over many years may break down owing to attack by specialised physiological races of pathogen. Tolerants are now being produced which, while biologically susceptible, are field resistant. Octoploid *Fragariae* species have been used extensively. (R. D. R.)

Laboratory method of assessing susceptibility of seedlings and runners to physiologic races of *P. fragariae* is described. Results obtained by selfing named varieties and wild species of *Fragaria* indicate that it may be possible to increase the resistance of commercial varieties to this disease. Inconsistencies obtained in degree of infection in some of the material tested are discussed. (I. G. M.)

IN- AND OUT-BREEDING IN HIGHER FUNGI

J. H. BURNETT
Department of Botany, St Salvator's College, University of St Andrews

Work carried out over the past few years at Liverpool and St Andrews on the mating systems of species of *Coprinus*, *Hypholoma*, *Nidularia*, *Polyporus*, *Polystictus* and *Varraria*, will be reviewed in connection with the following topics (*a*) numbers and stability of mating-type factors in natural populations ; (*b*) size of breeding populations and nature of the mycelium ; (*c*) genetically imposed inbreeding.

MATING-TYPE FACTORS OF *POLYSTICTUS VERSICOLOR*

M. PARTINGTON
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The physiological genetics of fungal mating systems are little investigated and poorly understood. Using the tetrapolar fungus *Polystictus versicolor* the effects of different mating-type factors, in compatible matings, on (*a*) hyphal fusion ; (*b*) nuclear migration ; and (*c*) clamp connection formation, were investigated. The results will be described and a suggestion made concerning a model for gene action in connection with the formation of clamp connections.

THE RELATIVE ABUNDANCE AND BALANCE OF SPECIES IN WILD POPULATIONS

C. B. WILLIAMS
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(Formerly Department of Entomology, Rothamsted Experiment Station, Harpenden, Herts)

The number of individuals in a species at any moment is a synthesis of all the effects of both biological and physical environment in the past, acting on the genetic make-up of that species. The relative abundance of all species in a wild population is the synthesis of all these syntheses, and through its very complexity, may become possible to analyse by statistical methods, particularly as there seems to be a general pattern common to most populations.

Samples from populations of many groups of animals, and insects, and also analyses of data from plant quadrats, have shown that in any wild population, consisting of a number of species, there are always more " rare " species with small numbers of individuals than " common " species with large numbers. The greater proportion of the individuals, however, belong to the few commonest species.

On an arithmetic scale the frequency distribution forms (in nearly all small samples) a hollow curve, which closely resembles a logarithmic series. For large samples, if the number of individuals per species are grouped on a geometric scale, the frequency distribution more closely resembles a " log-normal " distribution.

The sizes of genera, as measured by the number of species in each, also seem to follow the same general pattern.

Examples will be given and discussed. An attempt will be made to extend the reasoning to give a conceivable distribution in species of all the insects in the world. Also on the botanical side, to discuss the relation between area and number of species of flowering plants, from the smallest quadrats to the total area of the world.

ABSTRACTS of Papers read at the HUNDRED AND THIRTY-FIRST MEETING of the Society held on 13th and 14th NOVEMBER 1959, at UNIVERSITY COLLEGE, LONDON

A " LUXOID " MOUSE MUTANT WHICH LACKS THE SPLEEN

A. G. SEARLE
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Dominant hemimelia (*Dh*), 5 units from leaden in linkage group XIII, is very variable in the expression of its manifold effects. Heterozygotes may have a normal skeleton, but usually the pre-axial side of the hind-limb shows one or more of the following defects :—(i) elongation and triphalangy of the hallux, (ii) poly- or oligodactyly, often with syndactyly, (iii) tibial reduction, leading to fibular distortion and twisting of the leg, (iv) reduction of the femur, (v) reduction of the pubic element of the pelvic girdle. Homozygotes show severe pre-axial oligodactyly,

with loss of up to three digits ; hind-legs are small and twisted, with their skeletal elements greatly reduced. Fore-limbs are unaffected in both hetero- and homozygotes.

One constant visceral abnormality is of particular interest : both heterozygotes and homozygotes entirely lack the spleen. This happens even in some heterozygotes in which the skeleton is unaffected, suggesting that the spleen defect is a more basic one. Stomach size is also reduced, often very greatly in homozygotes, which also tend to lack the distal part of the gut. Homozygotes suffer from extreme hydronephrosis ; they may also lack the bladder and have a number of other visceral defects. They usually die a few days after birth.

The fertility of *Dh* heterozygotes seems unimpaired, despite the absence of a spleen. Preliminary results of developmental studies will be given.

PHYSIOLOGY AND BEHAVIOUR IN F_1 MICE SHOWING HETEROSIS

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F_1 mice derived from an A2G × C57BL cross have previously been shown to display greater heterosis in a cold environment than in a " normal " one (*J. exp. Genet.*, in press). Studies are now being made on various aspects of the physiology and behaviour of such heterotic mice. Traits which are being investigated include (*a*) aspects of " vigour " such as (i) activity, (ii) resting oxygen consumption and (iii) resistance to " stress " in the form of cold exposure, and (*b*) aspects of " energy " such as nest-building. Some account will be given of such observations as have yielded intelligible results by the date of the meeting.

GENETICS OF SOME BEHAVIOUR PATTERNS IN MICE

G. E. McCLEARN
Institute of Animal Genetics, Edinburgh, and University of California, Berkeley, U.S.A.

A programme of comparison of behaviour of inbred strains of mice has shown significant differences in learning efficiency, voluntary alcohol consumption, and locomotor activity.

In the latter characteristic, two sublines of the C57BL strain are highly active, and two sublines of the A strain are very inactive. Comparison of activity measures under white and under red illumination suggests that part of the phenotypic difference is attributable to differences of the visual mechanism between the strains. Maternal effect appears negligible in view of the essential identity of results on reciprocal F_1s between C57BL/10 and A/Jax.

F_1 and F_2 backcross generations derived from C57BL/Crgl and A/Crgl have been tested. With a square root transformation of data to equate parent strain variance, the F_1 variance significantly exceeds that of the parents. Average partial dominance in the direction of high activity is indicated in that the F_1 mean exceeds the midparent value, the F_2 mean is lower than the F_1 mean and the variance of the backcross to C57BL is less than that of the backcross to A. Comparing the arithmetic mean of the variances of parent strains and F_1 with the variance of F_2 suggests the degree of genetic control to be approximately 70 per cent.

OBSERVATIONS ON MUTANTS OF *ASPERGILLUS NIDULANS* INDUCED BY MONOCHROMATIC ULTRA-VIOLET LIGHT

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An account of mutants obtained by irradiating dry conidiospores in the Uvispek monochromator is given. Wave-lengths in the regions 265 and 280 m/u were

mainly used and absorption in the spore was measured by the Hilger photomultiplier and recorder. Efficiency of mutation production corresponded to the absorption curve of protein rather than nucleic acid, the mutants scored being (*a*) lethals, (*b*) abnormal growth patterns, (*c*) colour changes in conidia, (*d*) biochemical requirement.

EFFECTS OF SEED TREATMENT WITH IONISING RADIATION IN THE GROUNDSEL PLANT (*SENECIO VULGARIS* L.)

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Dry seeds of groundsel were irradiated in the Cobalt 60 source of the Christy Hospital, Manchester, at dosages from 500 to 100,000 r units. Germination of irradiated seeds gave no clear indication of any lethal effect below the 50,000 r level. One dwarf and sterile seedling in the 100,000 r group survived for 92 days. One small distorted seedling in the 75,000 r group survived for 120 days, producing a sterile flower.

The effect of radiation on fertility began at about the 30,000 r level. In this group more than 25 per cent. of the plants were sterile. Another 25 per cent. were of low fertility with less than 4 seeds per head.

At dosages of 20,000 r and below, the number of seeds per head was not significantly different from the control.

Progenies from all plants in the 30,000 r and 50,000 r groups were grown. No abnormalities appeared in the X_1 generation, and none were observed in X_2 progenies of the 50,000 r group. In the X_2 generation of plants of the 30,000 r group various recessive abnormalities occurred and are described. One abnormal form exhibited a type of blending inheritance.

The results are discussed in the light of changes in genetical architecture which might be expected to result from irradiation. It is considered that the most important consequence of irradiation is the production of numerous mutational changes in modifier or minor gene systems, *i.e.* in the general genetical background. This would lead to greater adaptational possibilities and would explain cases of blending inheritance.

STRUCTURE OF THE MATE-KILLING (MU) PARTICLES IN *PARAMECIUM AURELIA*, VARIETY 1

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An account will be given of studies on these *mu* particles by electron microscopy, fluorescence microscopy, and ordinary optical methods. The main conclusion is that the particles have a complex differentiated structure which will be described. They do not appear to be exactly like any known group of organism, but most nearly resemble certain bacteria.

GENETIC ANALYSIS OF SOME CONSEQUENCES OF DISRUPTIVE SELECTION

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Thoday and Boam (*Heredity*, *13*, 205 (1959)) by disruptive selection for sternopleural chaeta-number in *D. melanogaster* produced a population polymorphic for second chromosomes of low chaeta-number effect and second chromosomes of average chaeta-number effect.

We have investigated these second chromosomes with the following results. (1) The " low " second chromosomes are homozygous lethal. (2) Females made homozygous for an " intermediate " chromosome, when crossed to *y bw/bw st/st* males of a standard stock give progeny with a chaeta-number variance of 2 and a normal distribution, but females made heterozygous for two separately extracted intermediate chromosomes give progeny with a variance of 5 and a bimodal distribution, because recombination produces about 20 per cent. of " recombinant low " chromosomes. There is no sign of the expected reciprocal recombinant high class. (3) The " recombinant low " chromosomes prove homozygous lethal and lethal in combination with the " low " chromosomes originally extracted. (4) The original " intermediate " chromosomes can be classified into two classes $+-$ and $-+$ by such recombination tests. The low chromosomes are $--$. (5) Tests among the progeny of $+-/-+$ females have not so far demonstrated $++$ recombinants. It seems likely that they are dominant lethal.

GENE-FLOW AND DIVERGENCE UNDER DISRUPTIVE SELECTION

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Thoday and Boam (*Heredity*, *13*, 205 (1959)) have shown that two halves of a population between which there is maximal gene exchange (50 per cent. gene-flow) can diverge under disruptive selection. Random mating involves 25 per cent. gene-flow and we have therefore run two 25 per cent. gene-flow populations under disruptive selection with positive assortative mating and, for comparison, two "populations" with 0 per cent. gene-flow between the two halves which were selected in opposite directions for sternopleural chaeta-number (*D. melanogaster*). Divergence with 25 per cent. gene-flow was slower than with complete isolation but its ultimate magnitude in these very small populations was of the same order. A line taken from one of the 25 per cent. gene-flow populations and put under a regime involving 25 per cent. gene flow with negative assortative mating also maintained a large difference between its high and low halves. This regime is the exact equivalent of random mating between two populations selected in opposite directions in the pre-reproductive lives of the component individuals and is a clear demonstration that random mating cannot prevent evolutionary divergence. These populations are now being analysed genetically.

THE BREEDING STRUCTURE OF A LITTORAL POPULATION

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In three analyses of a wild population of *Coelopa frigida* (Fab.) several recessive egg lethal factors were found to be repeated. The repetition was found to be due both to method of sampling and to finite population size. The method of testing allelism of the lethals makes use of the fact that a female must copulate before each clutch of eggs is laid, and involves no dependance on visible markers. After correction for sampling method the gene frequency estimates for these loci were found to exceed the frequency expected on the basis of the census population size, N_0. From shifts in the lethal gene frequencies the size of the effective breeding unit N_e has been calculated. The ratio $N_e/N_0 = 0{\cdot}2$, shows that in this wild population a wide discrepancy exists between the observed and effective sizes. There is a non-Poisson variation in clutch size. An estimate of the reduction in size of the effective unit from this cause on a hypothesis of random progeny survival is small, but reduction on a hypothesis of family unit survival is large and approaches the estimate from frequency shifts. The ecological structure of the population strongly favours the latter type of survival.

A MECHANISM FOR INTER-ALLELE COMPLEMENTATION

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In *Neurospora crassa* several mutants, assignable to the *am* locus, lack detectable glutamic dehydrogenase, yet two kinds of heterocaryons formed from such mutants (am^1+am^2 and am^1+am^3) have this enzyme activity. In each case the enzyme formed has been shown to be qualitatively different from wild type glutamic dehydrogenase, and the enzymes in the two heterocaryons are different from each other.

It is suggested that there are probably two distinct kinds of complementary interaction between mutants which may be encountered in studies of the genetic control of the structure of single enzymes : (*a*) inter-locus interaction, in cases where the enzyme consists of non-identical sub-units (e.g. *Escherichia coli* tryptophan synthetase) ; (*b*) intra-locus interaction (complementation), which may be pictured as occurring where the enzyme consists of normally identical sub-units, with the possibility that sub-units which are the products of different mutant alleles, and which are defective in different respects, may polymerise together to give a complex with some activity. Interaction of type (*a*) is expected to result in qualitatively normal enzyme, that of type (*b*) in more or less abnormal enzyme.

FLARE REACTIONS TO INTRADERMAL INJECTIONS OF XANTHOSINE AND CHYMOTRYPSIN

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Intradermal injections of 5-10 micrograms of the nucleoside xanthosine, though painful to everybody, produced flares only in about half of 400 people of all ages. Reactions of xanthine but not to other nucleosides were perfectly correlated to the xanthosine reaction, and 24 individuals produced flares to xanthosine and the enzyme chymotrypsine, while 9 did not react to either. Mepyramine maleate, an antihistaminic suppressed flaring by xanthosine or chymotrypsin and prednisolone modified it. The skin reactions of a number of people, among which were sufferers from skin diseases, to acetyl choline, histamine and histamine releasing substances and to xanthosine were tested. In males flaring was a constant attribute of the individual but in some females it varied with the menstrual cycle. In spite of this difficulty in classification the following figures give some indication of the genetical situation ; all 16 children of 7 unions of non-reacting parents failed to produce flares ; 10 children of 5 reactor unions produced flares, but one, a son, did not ; of 13 children from mixed unions, 10 produced flares and 11 did not. This indicates a strong familial tendency for flaring ; formulation of a special genetical hypothesis will depend on improvements in female classification and on additional volunteers for the test.

HUMAN ISONIAZID METABOLISM. A STUDY OF THE GENETIC ASPECTS

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It has been shown that human subjects metabolise isoniazid in two different ways. " Slow inactivators " (S) have a gradual fall in free isoniazid concentration in the blood following an oral dose, and considerable amounts of the free drug appear in the urine. In " rapid inactivators " (R) there is a more rapid fall of blood concentration of free isoniazid, and a smaller amount appears in the urine after the same oral dose has been taken.

291 unrelated subjects have been examined. A single oral dose of 10 mg. isoniazid per kg. body weight was given and the plasma concentration of free isoniazid determined thereafter using a biochemical technique. All subjects were scorable into either S or R and the Caucasians were found to be about 50 per cent. S. The occurrence of these two phenotypes was shown not to be significantly different in 128 tuberculous patients and 91 American Negro subjects. A study of 53 Caucasian families has yielded data consistent with the hypothesis that the characters are controlled by a pair of autosomal allelic genes, the phenotype S being recessive.

EVIDENCE FOR A DISTURBED SEGREGATION OF ECTRODACTYLY IN MAN

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In a study of members of a large family with ectrodactyly first described by Karl Pearson in 1908, it was realised that the excess of affected in offspring over affected on a simple dominant hypothesis, noted by Pearson, persisted after a further three generations had been added to the family.

It was further remarked that this was explained entirely by an excess of affected males, and that this phenomenon only applied to the offspring of affected fathers and not to the offspring of affected mothers.

Study of the literature revealed a marked disturbance of sex ratio of affected and analysis of pedigrees where no generations were missed in manifestation of the abnormality, and where there were recorded inheritance through at least three generations, suggests strongly that the pattern in the Pearson family is not unusual. Summation of data shows an excess of affected males from affected fathers significant at a 1 per cent. level.

Analogies with the " T " allele system in mice are discussed and some thoughts engendered by presumptive evidence of disturbed segregation in man are presented.

ABO BLOOD GROUPS AND SECRETOR CHARACTER IN RHEUMATIC CARDITIS

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Data are presented of a series of 263 Liverpool patients suffering from rheumatic carditis. They show a significant reduction in the incidence of blood group O and a non-significant increase in non-secretors compared with controls. There is no heterogeneity with series of rheumatic fever patients from Taplow collected by Dr L. E. Glynn and his colleagues. Their data show a significant excess of non-secretors and a non-significant decrease in group O. On combining the results of the two investigations there is a significantly increased incidence of the disease both in those who are not group O ($\chi^2_1 = 8{\cdot}15$) and in those who are non-secretors ($\chi^2_1 = 6{\cdot}63$).

The levels of significance are very suggestive of there being a relationship between rheumatic fever and the ABO blood group antigens, but the collection of further data is indicated, not only of rheumatic fever but also of uncomplicated streptococcal throat infections and acute nephritis.